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HYBRID VIGOR IN SUGAR BEETS[1]

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INTRODUCTION

Hybrid vigor in first-generation crosses is a fundamental consideration in present-day methods of plant improvement. If it can be established that marked vigor rather generally occurs when inbred strains of sugar beet (*Beta vulgaris* L.) are crossed and that hybrids can be obtained which are superior to open-pollinated varieties, such findings would have important directive influence upon methods of sugar-beet breeding.

This report gives the results of field tests in which the sugar-beet hybrids arising as single crosses of inbred strains or as top crosses of inbred strains on an open-pollinated variety are compared with the parental sorts.

MATERIAL AND METHODS

The production by selection and inbreeding of the strains used in this study was begun by the late W. W. Tracy, Jr., in 1915 and continued by him until 1929, when the present writers took over the project. As a rule, a selfed generation was obtained every 2 years by bringing individual plants to seed either in home gardens some distance from each other or on widely separated farm locations. As the work proceeded, it was a general practice to test a number of sister progenies of a strain in each generation, discarding those considered undesirable. From the large collection of strains arising in Tracy's work, those listed in table 1 were chosen for crossings and tests for hybrid vigor. The original source or accession number of 1915 for a strain is given with the salient points of the breeding history. For easy reference in the text, the strains have been assigned Arabic numbers. Crosses between strains are designated in the customary manner, the seed-bearing parent being shown first.

To produce a hybrid, the two strains to be crossed were brought to seed production in a location at considerable distance from any other seed beets. The propensities of the unrelated strains to cross-pollinate reciprocally were relied upon to effect hybridization. In most cases the seed harvest was according to the strain serving as seed bearer, thus giving reciprocal crosses from each location. The sugar beet is known to be generally cross-pollinated under natural conditions, although selfing can be induced with a rather high percentage of plants taken from heterozygous populations. Completely self-sterile segregates or individuals that for other reasons fail to set seed drop out of the breeding material as inbreeding continues; hence, only lines with some degree of self-fertility were available in the breeding stock. In the progenies grown from the various hybrid

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seed lots there occurred not only F_1 plants but selfs of the strain that was seed bearer. Where distinctive characters were established in the parents, knowledge of the mode of inheritance was useful in identifying hybrid plants in the populations. In such cases the data are based upon the performance of the F_1 class of plants. With many of the progenies it has been necessary to include a considerable proportion of selfs; in others, the entire population, regardless of the actual extent of hybridization, has been taken. For this reason it is thought that in the considerations of performance in these cultures the comparisons with the strain serving as seed bearer are the most critical as well as the most conservative.

TABLE 1.—*Strains used in the production of hybrids, with salient points of their breeding history*

Designation of strain in text [1]	Generations		Seed lot used	Accession No. or source [2]	Designation of strain in text [1]	Generations		Seed lot used	Accession No. or source [2]
	Selfed	Grouped				Selfed	Grouped		
	Number	*Number*				*Number*	*Number*		
1	5	2	00569–0	1948.	9b	6	2	00203–0	1846.
2	6	2	4793–29	1846.	9c	7	1	10431–0	1846.
3	5	2	3444–29	1991.	10	5	1	10088s27	1899.
4a	5	2	506–28	2140.	11	3	3	3786–29	1736–6.
4b	4	3	4874–29	2140.	12	7	1	1223–29	280–32.
5a	6	2	6484–29	2073.	13	6	1	4563–27	2125.
5b	5	2	13844–27	2073.	14a	4	3	4842–29	1700–1.
6	7	1	1294–29	1892.	14b	5	2	6326–29	1700–1.
7	6	2	6571–29	2092.	15	1	0	11576s27	LSR.[3]
8a	5	3	2888–29	1910.	16	1	1	00383–0	La Royale.[4]
8b	5	3	3063–29	1910.	17	------	------	Pioneer	Commercial.[5]
9a	6	1	10742–27	1846.					

[1] Subgroups, representing different selections from essentially the same genetic complex, have been designated as a, b, or c.

[2] Strains 1 to 14, inclusive, trace to mass-selected breeding stocks received by W. W. Tracy, Jr., in 1915 from F. J. Pritchard, who had accumulated the material during his sugar-beet investigations. There was no history of inbreeding prior to Tracy's work.

[3] Leaf Spot Resistant, a variety developed by the agricultural research department of the American Crystal Sugar Co. under the supervision of Skuderna (*6*).[6]

[4] A commercial brand of K. Buszczynski & Sons, Ltd., Warszawa (Warsaw), Poland.

[5] A commercial brand of Zuckerfabrik Kleinwanzleben, Kleinwanzleben, Germany.

[6] Italic numbers in parentheses refer to Literature Cited, p. 738.

In obtaining the field data on performance, the roots were washed free of adhering soil, permitted to dry, and then weighed and counted to determine the average root weight. The roots were then individually split, one-half of each root being used in a composite plot sample for determining sucrose percentage in the pulp by the Sachs-Le Docte cold-water-digestion method. From the average root weight of a plot and the sucrose percentage of the plot sample, the average gross sugar per root was calculated for each plot. From the appropriate plot values, the mean for each strain or hybrid was obtained. For convenience in making comparisons of performance, the data for root weight and gross sugar have been calculated, by use of a factor for stand,[3] to show acre yields.

FIELD TESTS

For the field tests at Fort Collins, Colo., seed obtained from the various crosses and the parental strains was grouped according to

[3] 29,094, based upon stand counts of Pioneer for all tests.

relationship. There were 5 groups made up as shown in table 2 as tests 1, 2, 3, 4, and 5. The variety Pioneer,[4] commonly classed as a sugar type, was included in each test as a representative of non-inbred stock. The seed lot smallest in quantity determined the extent to which all within a given test could be planted. In test 1, all plots were 3 rows wide. It was necessary to restrict the plot width to a single row for 4 of the 6 replications in tests 3 and 4, and in 6 of the 8 replications in tests 2 and 5. Otherwise, three-row plots were used in these tests. Where single-row plots occurred, every alternate row was planted with Pioneer to give common competition. The experimental plots (26 feet long) were arranged for each of the 5 tests according to the equalized randomized-block scheme suggested by Student (*8*)[5] as a modification of the Latin square. The analysis of variance as given by Fisher (*4*) for a Latin square was used in statistical reduction of data. Shortly before harvest, the plants at the ends of each plot and those that bordered gaps along a row to be sampled were cut out. The remaining plants were harvested, only the center row in the three-row plots being used. In the case of a complete stand, there were 25 plants per plot; however, taking into account elimination of selfs and very occasional skips, the plots generally provided only 10 to 20 roots. The data for yield of roots, sucrose percentage, and yield of gross sugar as obtained in the tests are given in table 2.

For each of the economic attributes, insofar as comparisons can be made, the hybrid or the hybrid population plus selfs is compared with (1) mother strain, (2) pollen strain, (3) mean of both parents, and (4) the commercial brand, Pioneer. These comparisons are given as percentage values, where 100 is equality and values above or below represent performance either superior or inferior to the base chosen. The percentage values in terms of the performance of the commercial brand may be used as coefficients of performance for comparisons of strains or hybrids in one test with those occurring in another, since the commercial brand was included in each test.

CERCOSPORA LEAF SPOT

Late in July an epidemic of cercospora leaf spot (*Cercospora beticola* Sacc.) occurred and continued until harvest. This disease is known to influence the growth of the sugar beet, depressing root yield and sucrose percentage. Since the parental strains differed in resistance, a few being very susceptible, a factor of considerable importance was introduced. For certain crosses the response attributed to hybrid vigor would undoubtedly be less pronounced were tests conducted under disease-free conditions. Specific examples of crosses of this type are discussed later.

[4] One of the several commercial brands of foreign sugar-beet seed sold in this country at the time these investigations were initiated.

[5] Italic numbers in parentheses refer to Literature Cited, p. 738.

TABLE 2.—*Comparisons, in performance, of hybrids with their respective parental strains and with the commercial brand Pioneer[1] in tests at Fort Collins, Colo., 1932*

TEST 1. RESULTS BASED ON EIGHT-PLOT AVERAGES

Parent or hybrid	Yield of roots						Sucrose					Gross sugar					
	Average weight of root	Acre yield (calculated)[2]	Value expressed as percentage of—				Average in roots	Value expressed as percentage of—				Average weight per root[3]	Acre yield (calculated)[2]	Value expressed as percentage of—			
			Female	Male	Female + male / 2	Pioneer		Female	Male	Female + male / 2	Pioneer			Maternal strain	Pollen strain	Mean of both parents	Pioneer
Parents:	*Pounds*	*Tons*					*Percent*					*Pound*	*Pounds*				
Strain 1	0.9574	13.93					14.05					0.13433	3,908				
Strain 9a	.9311	13.54					11.10					.10333	3,006				
Pioneer (17)	.9305	13.54					13.00					.12092	3,518				
Hybrids:[4]																	
1 (row 1) × 9a	1.6593	24.14	173	178	176	178	13.08	93	118	104	101	.21697	6,313	162	210	183	179
1 (row 3) × 9a	1.4900	21.68	156	160	158	160	13.34	95	120	106	103	.19789	5,757	147	192	167	164
1 (row 5) × 9a	1.5029	21.86	157	161	159	162	13.14	94	118	104	101	.19712	5,735	147	191	166	163
1 (row 7) × 9a	1.3538	19.69	141	145	143	145	14.13	101	127	112	109	.19106	5,559	142	185	161	158
1 (row 9) × 9a	1.5880	23.10	166	171	168	171	13.41	95	121	107	103	.21307	6,199	159	206	179	176
1 (row 11) × 9a	1.5688	22.82	164	168	166	169	13.06	93	118	104	100	.20461	5,953	152	198	172	169
Average	1.5271	22.22	160	164	162	164	13.36	95	120	106	103	.20346	5,919	151	197	171	168
$9a_1 \times 1$	1.3403	19.50	144	140	142	144	12.32	112	88	99	95	.16541	4,812	160	123	139	137
$9a_2 \times 1$	1.6126	23.46	173	168	171	173	12.49	113	89	99	96	.20219	5,883	196	151	170	167
$9a_3 \times 1$	1.5890	23.12	171	166	168	171	12.51	113	89	99	96	.19904	5,791	193	148	168	165
$9a_4 \times 1$	1.7129	24.92	184	179	181	184	12.45	112	89	99	96	.21325	6,204	206	159	179	176
$9a_5 \times 1$	1.5124	22.00	162	158	160	163	12.80	115	91	102	98	.19192	5,584	186	143	162	159
Average	1.5534	22.60	167	162	165	167	12.53	113	89	100	96	.19436	5,655	188	145	164	161
1 × Pioneer (17)	1.1714	17.04	122	126	124	126	14.58	104	112	108	112	.17037	4,957	127	141	134	141
Pioneer (17) × 1	1.0423	15.16	112	109	110	112	13.84	106	99	102	106	.14399	4,189	119	107	113	119
Average	1.1068	16.10			117	119	14.21			105	109	.15718	4,573			123	130
Difference required for significance[5]	.2100	3.05					.45					.02784	810				

TEST 2. RESULTS BASED ON EIGHT-PLOT AVERAGES

Parents:																	
Strain 1	0.7195	10.47					14.90					0.10701	3,113				
Strain 9a	.6144	8.94					11.93					.07355	2,140				
Strain 9b	.7546	10.98					12.95					.09518	2,769				
Strain 11	.5825	8.47					13.84					.08002	2,328				
Strain 16	.7751	11.28					14.66					.11320	3,293				
Pioneer (17)	.8199	11.93					13.60					.11070	3,221				
Hybrids:																	
1 × 9a	1.4098	20.51	196	229	211	172	13.70	92	114	102	101	.19273	5,607	180	262	213	174
1 × 9b	1.3960	20.31	194	185	189	170	14.20	95	110	102	104	.19767	5,751	185	208	196	179
Reciprocal	1.3586	19.76	180	189	184	166	13.75	106	92	99	101	.18649	5,426	196	174	184	168
1 × 17	1.2021	17.49	167	147	156	147	14.60	98	107	103	107	.17550	5,106	164	159	161	159
11 × 1	1.0370	15.09	178	144	159	126	13.76	99	92	96	101	.14297	4,160	179	134	153	129
11 × 9a	1.3290	19.33	228	216	222	162	14.16	102	95	110	104	.18867	5,489	236	257	246	170
Reciprocal	1.7774	25.86	289	305	297	217	14.13	118	102	110	104	.24985	7,296	340	312	325	226
11 × 17	.8878	12.91	152	108	127	108	14.00	101	103	102	103	.12366	3,598	155	112	130	112
Reciprocal	.8338	12.13	102	143	119	102	14.08	104	102	103	104	.11711	3,407	106	146	123	106
16 × 11	.9336	13.58	120	160	138	114	14.00	96	101	98	103	.13028	3,790	115	163	135	118
Difference required for significance [5]	.1829	2.66					.57					.02456	713				

TEST 3. RESULTS GIVEN AS SIX-PLOT AVERAGES

Parents:																	
Strain 1	0.7280	10.59					15.03					0.10930	3,180				
Strain 2	1.0188	14.82					14.75					.14969	4,355				
Strain 4b	.8927	12.99					14.27					.12719	3,700				
Strain 7	.8523	12.40					15.18					.12919	3,759				
Strain 8a	.8468	12.32					13.85					.11726	3,412				
Strain 15	.9640	14.02					13.72					.13293	3,867				
Pioneer (17)	.8205	11.94					13.60					.11150	3,244				
Hybrids:																	
(1 × 2) [6]	1.1662	16.96			134	142	14.45			97	106	.16810	4,891			130	151
1 × 2	1.3037	18.96	179	128	149	159	14.43	96	98	97	106	.18835	5,480	172	126	145	169
Reciprocal	1.0463	15.22	103	144	120	128	14.00	95	93	94	103	.14551	4,233	97	133	112	131
1 × 8a	1.2127	17.64	167	143	154	148	14.73	98	106	102	108	.17899	5,208	164	153	158	161
1 × 15	1.0772	15.67	148	112	127	131	13.98	93	102	97	103	.15070	4,384	138	113	124	135
4b × 1	.9972	14.51	112	137	123	122	14.67	103	98	100	108	.14596	4,247	115	134	123	131
4b × 15	1.2753	18.55	143	132	137	155	13.75	96	100	98	101	.17527	5,099	138	132	135	157
7 × 1	1.1888	17.29	139	163	150	145	13.77	91	92	91	101	.16371	4,763	127	150	137	147
7 × 8a	1.2015	17.48	141	142	141	146	11.98	79	87	83	88	.14336	4,171	111	122	116	129
Reciprocal	1.2938	18.82	153	152	152	158	11.85	86	78	82	87	.15297	4,451	130	118	124	137
8a × 7	1.1413	16.60	135	134	134	139	12.85	93	85	89	95	.14654	4,263	125	113	119	131
Difference required for significance [5]	.1528	2.22					.52					.02184	635				

See footnotes at end of table.

TABLE 2.—*Comparisons, in performance, of hybrids with their respective parental strains and with the commercial brand Pioneer*[1] *in tests at Fort Collins, Colo., 1932*—Continued

TEST 4. RESULTS GIVEN AS SIX-PLOT AVERAGES

Parent or hybrid	Yield of roots						Sucrose					Gross sugar					
	Average weight of root	Acre yield (calculated)[2]	Value expressed as percentage of—				Average in roots	Value expressed as percentage of—				Average weight per root[3]	Acre yield (calculated)[2]	Value expressed as percentage of—			
			Female	Male	Female + male / 2	Pioneer		Female	Male	Female + male / 2	Pioneer			Maternal strain	Pollen strain	Mean of both parents	Pioneer
Parents:	*Pounds*	*Tons*					*Percent*					*Pound*	*Pounds*				
Strain 1	0.7582	11.03					15.08					0.11399	3,316				
Strain 5a	.9797	14.25					14.90					.14599	4,247				
Strain 8b	.9888	14.38					14.12					.13936	4,055				
Strain 10	.9498	13.82					13.73					.13024	3,789				
Strain 14a	.7618	11.08					13.90					.10561	3,073				
Strain 14b	.7772	11.31					13.43					.10438	3,037				
Pioneer (17)	.8355	12.15					13.88					.11596	3,374				
Hybrids:																	
1 × 5a	1.3822	20.11	182	141	159	165	14.65	97	98	98	106	.20149	5,862	177	138	155	174
1 × 5a	1.5892	23.12	210	162	183	190	13.70	91	92	91	99	.21644	6,297	190	148	167	187
5a × 10	.8882	12.92	92	94	92	106	15.02	101	109	105	108	.13324	3,876	91	102	96	115
Reciprocal	1.0645	15.49	112	109	110	127	14.10	103	95	99	102	.15035	4,374	115	103	109	130
8b × 5a	.9173	13.34	93	94	93	110	14.06	99	94	97	101	.12837	3,735	92	88	90	111
8b × 1	1.3018	18.94	132	172	149	156	14.13	100	94	97	102	.18330	5,333	132	161	145	158
(8b × 10)[6]	1.0208	14.85			105	122	13.07			94	94	.13332	3,879			99	115
8b × 14b	1.0173	14.80	103	131	115	122	14.27	101	94	104	103	.14508	4,221	104	139	119	125
10 × 14b	.9555	13.90	101	123	111	114	13.48	98	100	99	97	.12879	3,747	99	123	110	111
Reciprocal	1.0020	14.58	129	105	116	120	12.87	96	94	95	93	.12796	3,723	123	98	109	110
14a × 1	.9215	13.41	121	122	121	110	13.53	97	90	93	98	.12483	3,632	118	110	114	108
Difference required for significance[5]	.1920	2.79					.58					.02530	736				

TEST 5. RESULTS GIVEN AS EIGHT-PLOT AVERAGES

Parents:																		
Strain 1	0.7843	11.41					14.25					0.11146	3,243					
Strain 3	.8180	11.90					15.15					.12390	3,605					
Strain 4a	1.0491	15.26					12.30					.12792	3,722					
Strain 5b	1.0655	15.50					13.08					.13936	4,055					
Strain 6	1.2258	17.83					13.39					.16318	4,748					
Strain 9c	.7228	10.51					12.58					.09065	2,637					
Strain 12	1.1670	16.98					13.16					.15342	4,464					
Strain 13	.9975	14.51					11.61					.11560	3,363					
Pioneer (17)	.9441	13.73					13.46					.12720	3,701					
Hybrids:[6]																		
(1 × 3)	1.1203	16.30			140	119	14.28			97	106	.15730	4,576			134	124	
(1 × 6)	1.3939	20.28			139	148	13.21			96	98	.18401	5,354			134	145	
(1 × 6)	1.3055	18.99			130	138	13.34			97	99	.17465	5,081			127	137	
(1 × 12)	1.3241	19.26			136	140	13.44			98	100	.17829	5,187			135	140	
(4a × 5b)	1.1073	16.11			105	117	12.96			102	96	.14318	4,166			107	113	
(4a × 12)	1.2975	18.87			117	137	12.35			97	92	.15989	4,652			114	126	
(5b × 9c)	1.3736	19.98			154	145	12.63			95	94	.17392	5,060			151	137	
(6 × 9c)	1.3990	20.35			144	148	12.00			92	89	.16677	4,852			131	131	
(6 × 13)	1.2093	17.59			109	128	11.35			91	84	.13502	3,928			97	106	
Difference required for significance [5]	.1778	2.59					.56					.02197	639					

[1] Produced by the Zuckerfabrik Kleinwanzleben, Kleinwanzleben, Germany.

[2] Calculated from average individual root values to acre basis by assuming a stand per acre of 29,094 beets. This value for stand was obtained by averaging the stand of all check plots in the field.

[3] Obtained by averaging individual plot values, hence differing slightly from products of means for average root weight and sucrose percentage shown.

[4] Progenies of sibs of strain 9a were the respective seed bearers in this series of crossings and are designated by the subscript numerals 1, 2, 3, etc. Seed from strain 1 was kept separate by rows.

[5] Twice the standard error of the difference of 2 means.

[6] Seed from strains entering the cross pooled.

RECIPROCAL CROSSES OF STRAINS 1 AND 9a

The single cross between strains 1 and 9a was considered very desirable for a detailed study of the vigor of hybridity in the sugar beet. In previous tests each inbred had shown striking phenotypic uniformity (fig. 1). Numerous progenies of each had been grown and studied under field conditions. These always adhered closely to the parental strain type, predicating an approach to homozygous condition

FIGURE 1.—Four-row plots of strain 1 (*A*) and strain 9a (*B*) grown at Fort Collins, Colo., in 1929. Strain 9a is susceptible to cercospora leaf spot, as evidenced by the dead outer leaves.

for genes determining major morphological characters. The plants of strain 9a used for seed production were sib progenies of the strain, each progeny forming a separate row. The plants of strain 1 were set in rows alternating with strain 9a. The seed produced was harvested separately from each row. In all, 11 seed lots were obtained, 6 from the various rows made up of strain 1, and 5 from the sister progenies of strain 9a (designated as $9a_1$, $9a_2$, $9a_3$, etc.). These seed lots were used in test 1, and the results are given in the first section of table 2.

Each parental strain was distinct from the other. The difference in length and type of petiole, shape of leaf, and shade of green color,

associated with a peculiar habit of growth, gave a striking contrast in the foliar bouquet of the two strains (fig. 2). In populations arising from the reciprocal crosses, the foliage as well as the root type was used to distinguish between inbred and hybrid classes. All plants

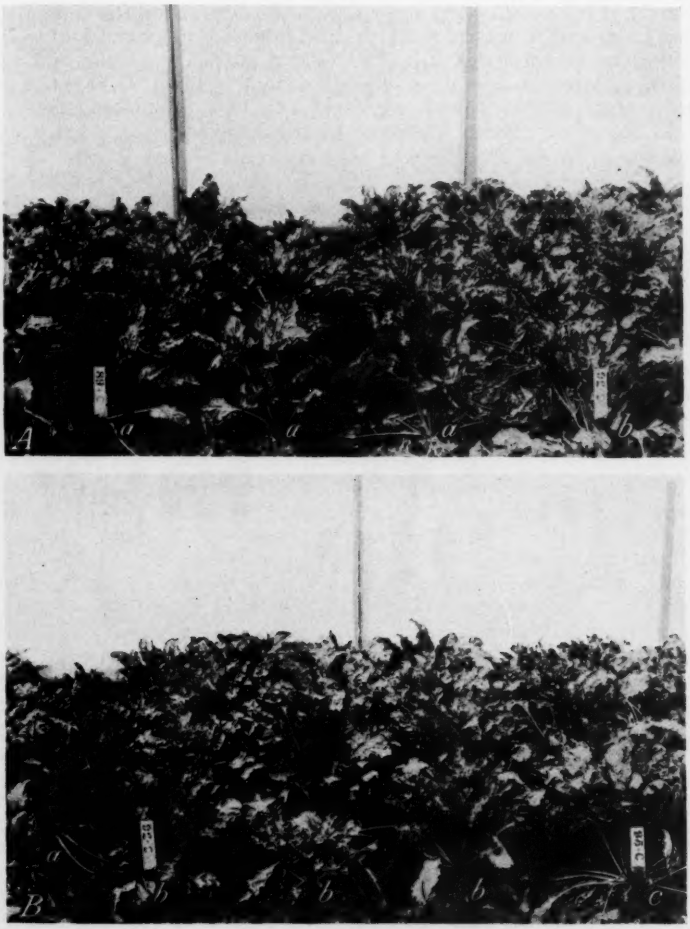

FIGURE 2.—Field view of strains 1 and 9a and the hybrid 1×9a, grown at Fort Collins, Colo., 1932, showing greater foliage growth and vigor of the hybrid: *A*, Three-row plot of strain 9a (*a*) and of the hybrid 1×9a (*b*); B, strain 9a (*a*), three-row plot of the hybrid 1×9a (*b*), and strain 1 (*c*).

that were judged to have originated by selfing were eliminated; however, in case a plant could not be classified with sureness it was always included in the sample as a hybrid. The data as reported are considered to represent largely the performance of the hybrid class of the population.

The parental strains 1 and 9a had average root weights of 0.9574 and 0.9311 pound, respectively, indicating that under the conditions they were very similar in growth capacity. The hybrids 1 (rows 1, 3, 5, 7, 9, 11)×9a had an average root weight of 1.5271 pounds; the hybrids 9a (progenies 1–5)×1 had an average root weight of 1.5534 pounds. The striking vigor of the hybrid is shown by the comparison of average root weight of the 11 hybrid lots with the mean root weight of the two parents (fig. 3). The hybrid gave an average of 1.5391 pounds, corresponding to a calculated acre yield of 22.41 tons; and the parents, 0.9443 pound, corresponding to a calculated acre yield of 13.735 tons. When expressed as percentage of the mean weight

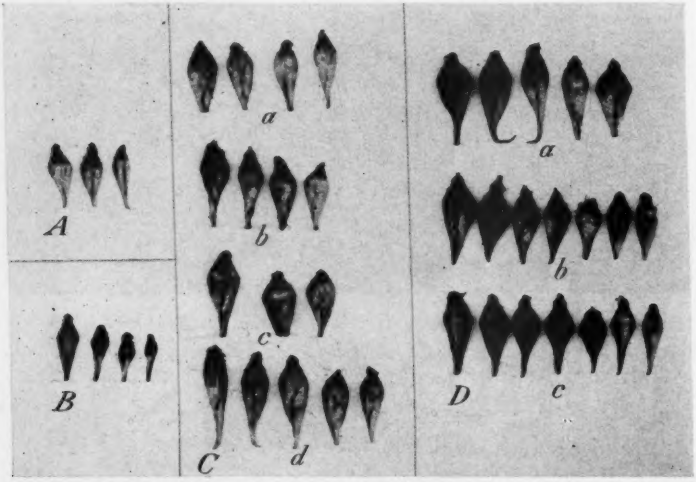

FIGURE 3.—Typical roots of the parental strains 1 (*A*) and 9a (*B*) and their hybrids, as obtained in test 1, Fort Collins, Colo., in 1932. *C*, Hybrid 1×9a, roots grown from seed harvested from strain 1 as seed bearer; *a*, *b*, *c*, *d*, seed taken from rows 1, 3, 9, and 11, respectively. *D*, Hybrid 9a×1, roots from the reciprocal cross, in which sibs 2, 5, and 7 (*a*, *b*, *c*) of strain 9a were the seed bearers.

of the parents, the increase of the hybrid is approximately 63 percent. Since the parental strains were similar in yield, the hybrid exceeded each by approximately the percentage as found for the mean of the parents.

Previous tests had shown the parent strains to be distinctly unlike in richness of sucrose. The mean sucrose percentages obtained in this test, 14.05 for strain 1 and 11.10 for strain 9a, are in agreement with those obtained formerly. The sucrose percentages as shown in table 2 for this hybrid in all cases were higher than the low-sucrose parent, and in all but one case lower than the high-sucrose parent. The mean value for the hybrid approximated the mean of the parents. The hybrid populations in table 2 representing the cross 1 × 9a, in which the strain higher in sucrose percentage was the maternal parent, were consistently higher in sucrose than the populations in the other group, resulting from the reciprocal cross, in which the

strain lower in sucrose was the seed bearer. The difference in sucrose percentage between the reciprocals is statistically significant. Bougy (*1*), in controlled crosses of sugar beets and forage beets (Vauriac), did not obtain perfect reciprocity for sucrose percentage. However, it seems justifiable to assign the difference in these comparisons of reciprocals to incomplete elimination of selfs, which would tend to shift the readings in the direction of the respective maternal parents rather than to postulate a specific maternal influence. Savitsky (*5*) reports that in crosses between varieties of high- and those of low-sucrose percentage the F_1 generation was, in this respect, close to the arithmetical average of the parents.

The total sucrose in the roots, which is the product of root weight and sucrose percentage, was markedly increased in the hybrid over that of either parent. Since the average sucrose percentage of the hybrid approximated the mean value of the parents, this increase may be attributed almost wholly to the greater root size of the hybrid. The percentage increase (67 percent) over the mean of the parents is about in the proportion of the relative root weights.

The increase in root weight assignable to hybrid vigor in this cross far exceeds the statistical requirement for significance. The difficulties of excluding all selfs have been discussed in connection with the sucrose percentage of the hybrid. It is to be noted that in the test of these reciprocal crosses seed produced from six groups or rows of strain 1 flanked by rows of sib progenies of strain 9a was used. There is a remarkable concordance of performance among these different representatives of the mating. The results show clearly that, regardless of the direction of the cross, the hybrid was significantly greater in weight than either parent. From this, it is concluded that the vigor of the hybrid is not merely the expression of dominance of some simple character possessed by one parent but is of the same nature as heterosis response demonstrated for other plants.

HYBRIDS INVOLVING MANY STRAINS

In tests 2, 3, 4, and 5, as shown in table 2, an attempt was made to include crosses that involved a wide range of parental types. Inbred strains known to possess important characters such as high sucrose percentage or disease resistance were more generally used in the pairings. To only a limited extent could reciprocal crosses of a given hybrid be included in the test, as deficiency in quantity or quality of seed obtained from one strain entering the cross commonly made omissions necessary. Crosses in which the seed produced by both parental strains was harvested as one lot were also included in the tests. Although seed lots obtained in this manner are not so serviceable for interpretation of hybrid vigor as those harvested separately for each strain entering a cross, their inclusion is considered of value because of the indication obtained of the type of performance to be expected if the two strains are interplanted for extensive seed production of a first-generation hybrid. The results of the 4 field tests, involving in all 41 hybrid populations, the respective parents, and the commercial brand, are given in table 2.

The data are first discussed with general regard to the hybrid vigor resulting from crosses between sugar-beet strains; then the response of individual strains when hybridized is considered because of indications given as to their possible breeding value.

Root Weight of Hybrids

As mentioned earlier, the comparison of the hybrid with its maternal strain, because of the incomplete elimination of selfs that can be effected at harvest, is probably the most conservative measure of performance. In such a comparison, however, as has been noted with other crop plants, the percentage increase in the yield of the hybrid over that of a low-yielding parent may be strikingly greater than the percentage found if the maternal parent itself is relatively high in yield.

In 30 of the 41 crosses the seed had been harvested separately for each strain entering a cross. The average root weights of the plants grown from hybrid seed were significantly greater than the average root weights of the respective maternal parents in 20 of the 30 crosses. In 10 cases significant differences between the hybrid and the maternal strain were not found. For the 30 comparisons the general average root weight showed an increase of 50 percent for the populations arising from these crosses over the general average root weight of the maternal parents.

The comparisons in terms of the mean of parents are also of interest, since all hybridizations can be included. In 31 of the 41 cases the root weights of the hybrids were significantly greater than the means for root weight of the appropriate parent strains; significant differences were not indicated in 10 cases, although in one cross 14b $\times$ 10 the hybrid had been significantly superior to the maternal strain. The general average root weight of all hybrids exceeded the general average root weight of parents by at least 42.5 percent. In the consideration of the foregoing data, attention is again called to the fact that elimination of selfs was incomplete in many cultures.

The average root weight of the 41 hybrids was found to be 38.9 percent greater than that of the commercial brand, Pioneer. On the basis of extensive tests with commercial brands, among which Pioneer was included as a representative of sugar types, Skuderna et al. (7) found that as an average the sugar types were exceeded in root weight by yield types by approximately 10 percent. On such a basis it would seem that in acre yield of roots the hybrids as a whole compare very favorably with commercial yield types and that certain hybrids far exceed them.

Sucrose Percentage of Hybrids

For the 30 crosses in which the seed was kept separate as to the seed-bearing strain, it was found that in 17 cases the sucrose percentage of the hybrid was not significantly different from that of its maternal strain; in 2 cases it was significantly superior, and in 11 cases significantly inferior. The general average sucrose percentages for this entire group did not differ significantly from the mean of all strains involved as seed bearers.

The average sucrose percentage of the 41 hybrid populations, although slightly lower, did not differ significantly from the mean of the parental strains.

Twenty-three of the forty-one hybrids were not significantly different in sucrose percentage from the commercial brand, Pioneer. Nine were significantly less and nine significantly higher. The average sucrose percentage of the 41 hybrids equaled that of Pioneer.

This indicates, on the whole, a very satisfactory richness in sucrose for hybrid beets.

Total Sucrose in Hybrids

Hybrid vigor was very pronounced in many of these crosses, increasing root weights significantly over the root weights of the parents. Since the sucrose percentages found approximated, as an average, the mean sucrose percentage of the parents, the total sugar produced was higher in the majority of cases than the mean sugar production found for the parental strains, this being assignable to the increased root yield of the hybrids.

Similarly, in the comparison of the hybrids as a whole with the commercial brand, since the average sucrose percentages of the hybrids and of Pioneer were approximately the same, sugar production of the hybrid populations exceeded that of the commercial brand by about the percentage found in the comparisons of root weight (39 percent).

Response of Individual Strains When Hybridized

strain 1

Strain 1, which is discussed on page 722, occurred as a parent in 20 of the 41 hybrid cultures whose performance data are given in table 2. Field views showing one-row plots of various hybrids in which strain 1 occurred as a parent are shown in figure 4. In four instances the crosses were in duplicate. For the crosses with strain 2 and strain 9b, seed was available for test of reciprocals. The hybrid $1 \times 9a$, which occurred in test 2, has also been considered in the discussion of test 1.

In eight cases (tests 2, 3, and 4, table 2) seed was harvested from strain 1 as seed bearer. The performance data shown for these hybrids are based largely upon the F_1 portion of the progeny, since the rather distinctive selfs of strain 1 could be eliminated. For each F_1 progeny, the yield of roots is significantly above that of the maternal strain, above that of the pollen parent except in one instance, and above the mean root yield of the parental strains. The average percentage increase in root weight of the hybrids, found by averaging the appropriate individual percentages given in table 2, is 80.4 percent over the average root weight of maternal strains and 66.0 percent above the mean root weight of both parents. It also exceeds the average root weight of Pioneer by 60.3 percent.

Strain 1 occurs as pollen parent in seven crosses. The performance data for these are based upon mixed populations of hybrids except in the progenies from crosses $9b \times 1$ and 11×1 (test 2, table 2). Each of the seven progenies from hybridization gave a larger root yield than either parent, the increase in root yield over that of the respective maternal strain being statistically significant in four cases; the root yields were significantly greater than the yields of the pollen parent in 6 cases out of the 7, and accordingly exceeded the mean yields of both parents. The average percentage increases in root yield for these seven populations containing hybrids are 38 percent above that of both parents and 36.3 percent above that of the commercial brand Pioneer.

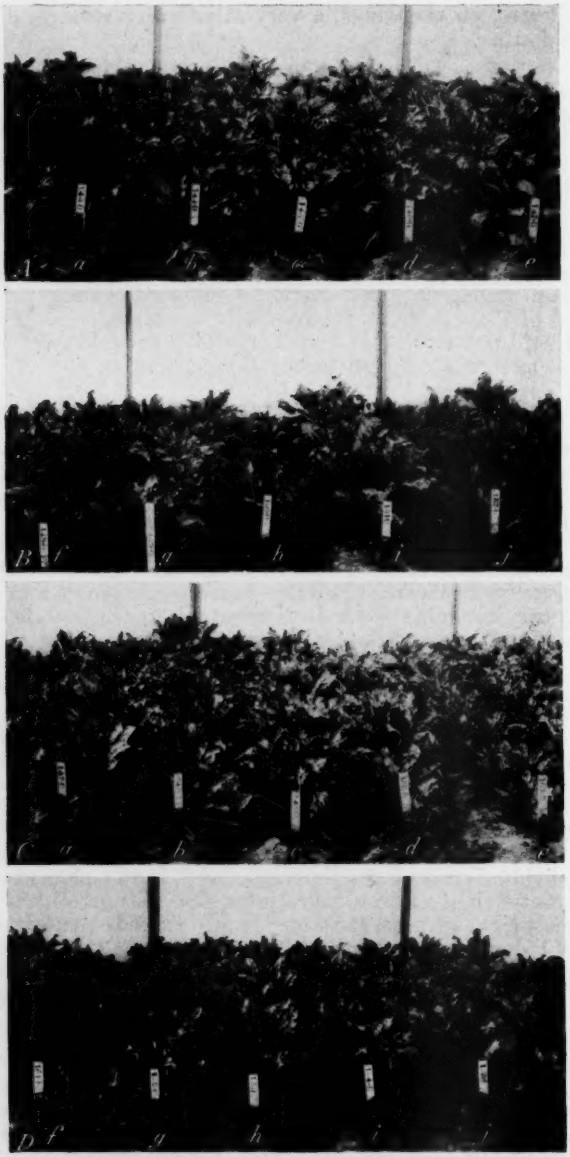

FIGURE 4.—Field views of one-row plots of two replications of test 2. Alternate plots planted with Pioneer to give common competition. *A* and *B*, Plots of D series: *a*, Strain 1; *b*, Pioneer; *c*, strain 16; *d*, Pioneer; *e*, hybrid 11×1; *f*, Pioneer; *g*, hybrid 16×11; *h*, Pioneer; *i*, hybrid 11×9a; *j*, Pioneer. *C* and *D*, Plots of E series: *a*, Hybrid 9a×11; *b*, Pioneer; *c*, hybrid 1×9a; *d*, Pioneer; *e* and *f*, strain 9a; *g*, Pioneer; *h*, strain 11; *i*, Pioneer; *j*, strain 1.

Strain 1 also occurred as a parent in five crosses in which the seed produced by the two strains entering a cross was pooled at harvest. The progenies obtained consisted not only of F_1 generations but selfs of each parental strain. Comparison of root weight with the mean root weight for the appropriate parent strains and for Pioneer can be made. In each cross, regardless of inclusion of selfs, the populations obtained were significantly superior in root yield and in sugar production to the mean of parents and to Pioneer, the average percentage increase being, for each basis, approximately 34 percent. Superiority in sugar production was in about the same proportion.

In general, the result of crossing strain 1 with 15 unrelated sorts was a marked increase in vigor in the first-generation hybrid (fig. 5, *A, B, C, D*). The preponderance of evidence indicates reciprocity of the effect of hybridity when reciprocals are compared, or when the yields of hybrids in which strain 1 is the pollen parent are considered in terms of root yields of the respective mother strains.

STRAIN 2

Strain 2, whose pedigree shows six generations of selfing, interspersed with 4 years of group increase, has been characterized by heavy foliage production and exceptionally high root yields. It has shown moderately high sucrose percentage and moderate leaf spot resistance. In test 3 (table 2) it was used only in crosses with strain 1. Data are available for this cross from progenies of seed harvested from each parent as a seed bearer, and from a progeny in which seed from both parents was pooled.

In the cross 1×2, the hybrid was superior in performance to either parent. In the reciprocal, a gain over the maternal parent was shown, but this, however, did not reach significance. The hybrid progeny grown from the pooled seed gave an acre yield of roots of 16.96 tons. The mean acre yield of roots of the reciprocals was 17.09 tons, an entirely concordant value, which exceeds significantly the mean root yield of the parents.

The divergence in root yields in the reciprocals seems attributable to difference in harvest methods employed. In the plots of the cross 1×2, it was possible to exclude selfs rather rigorously, while for the plots of the reciprocals samples taken were not so restricted. The hybrid 2×1 did not exceed significantly the root weight found for strain 2. Since strain 2 is not known to be strongly self-fertile, it is to be presumed that the progeny contained a fair proportion of hybrids. It is known for other crops that the heterosis effect is less strikingly shown by vigorous inbred strains.

STRAIN 3

Strain 3 is characterized by dark-colored foliage, medium root weight (about equal to that of Pioneer), and high sucrose percentage. In leaf spot resistance it ranks above strain 2, but is less resistant than strain 1. Although it has since been extensively used in crossing, in these tests it was represented as a cross with strain 1 (test 5, table 2) in which the seed was not kept separate as to seed bearers (fig. 5, *C*). The performance of this hybrid indicated a root yield 40 percent above the mean of the two parents; the hybrid apparently is significantly above Pioneer in root yield. Sucrose percentage was high and approximated that of strain 1.

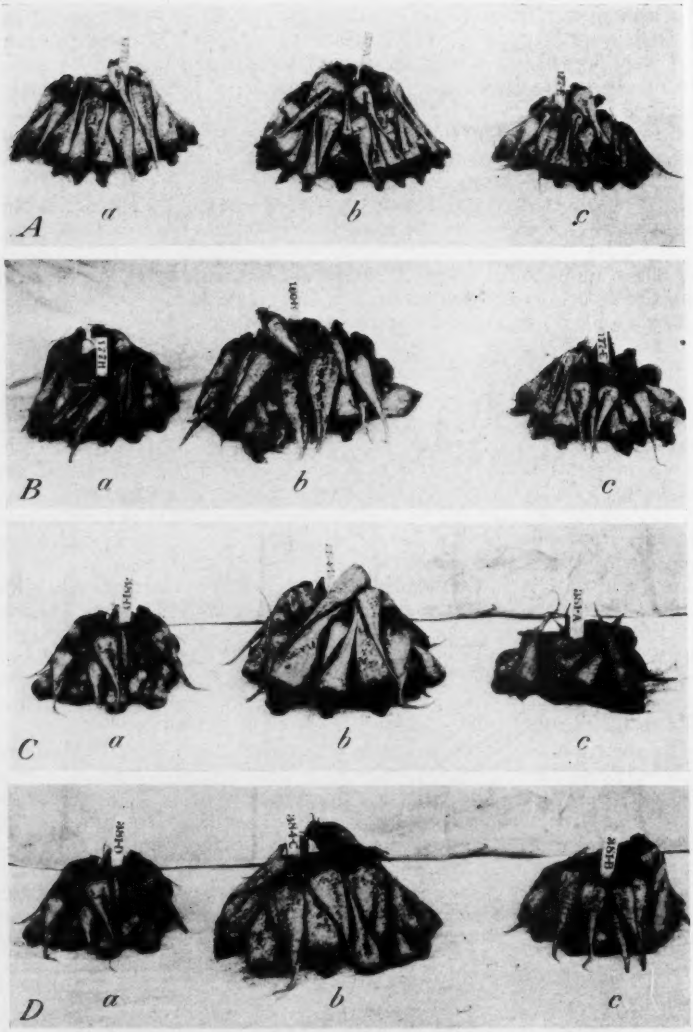

FIGURE 5.—Sugar-beet roots from plots planted with parental strains and with seed obtained by interplanting strains. (Roots taken from border rows of the plots but representative of roots from which data were obtained.) *A*, Strain 5a (*a*), hybrid 1×5a (*b*), and strain 1 (*c*); test 4. *B*, Strain 7 (*a*); hybrid 7×1 (*b*), strain 1 (*c*); test 3. *C*, Strain 1 (*a*), hybrid (1×3) (*b*), and strain 3 (*c*); test 5. *D*, Strain 1 (*a*), hybrid (1×6) (*b*), and strain 6 (*c*); test 5.

STRAIN 4

Strain 4 is represented by separate substrains, a and b, having essentially the same pedigree and close similarity in habit. The strain has been under observation since 1926 because of strong resistance to *Cercospora beticola*. Strain 4b was used as the seed bearer in crosses with strains 1 and 15. The hybrid 4b × 1 did not give a root yield significantly above that of the mother strain; the root yield of the hybrid was apparently superior to the mean of the two parents. The hybrid 4b × 15 gave a root yield which was significantly above that of either parent. In the latter cross, no attempt was made to separate the progeny into hybrid and selfed classes, nevertheless the root yield attributed to the hybrid was 43 percent above that of substrain 4b and 37 percent above the mean root yield of the parent strains. The root yield of Pioneer was exceeded by the hybrid by 55 percent. The occurrence of an epidemic of cercospora leaf spot has been mentioned previously. In these tests, the hybrid 4b × 15 was strikingly resistant, whereas Pioneer was susceptible. The ratio of performance with respect to Pioneer was decidedly influenced by the differences in disease reaction. However, the disease influence was not a factor of importance in the comparative vigor of the cross and parents, since the strains crossed are among the most resistant inbreds in the test. Strain 4a was crossed with strain 5b and strain 12, respectively, the seed from the seed bearers being pooled in the case of each hybridization. In the cross 4a × 5b, the root weight of the hybrid was only slightly above the mean root weights of the strains entering the cross or of Pioneer. The hybrid 4a × 12 was found to produce a root weight not significantly superior to the parental mean for root weight (fig. 6, *A*). It exceeded the commercial brand in root weight by 37 percent.

STRAIN 5

Strain 5, which is characterized by strong cercospora leaf spot resistance and a high degree of uniformity, was used as substrains a and b, which differed slightly in pedigree but represented selections out of the same genetic complex. Seven crosses were made using strain 5 (either a or b) with strains 1 (in duplicate), 4a, 8b, 9c, and 10 (reciprocal). The hybrids 1 × 5a and 4a × 5b have already been discussed. In performance, the hybrids were somewhat inconsistent, which may indicate that strain 5 is restricted in its value as breeding material. In the case of the cross with strain 10, in which reciprocals were tested, root yields differed rather widely but probably not significantly. The hybrid 10 × 5a exceeded Pioneer significantly in root yield but not its parents. The hybrid 5b × 9c exceeded in root weight the mean for root weight of the parents and significantly outyielded the commercial brand, Pioneer, as well.

UTILIZATION OF STRAINS 1 TO 5

A detailed discussion has been given of strains 1 to 5 and their behavior in hybridizations because these strains, on the basis of this

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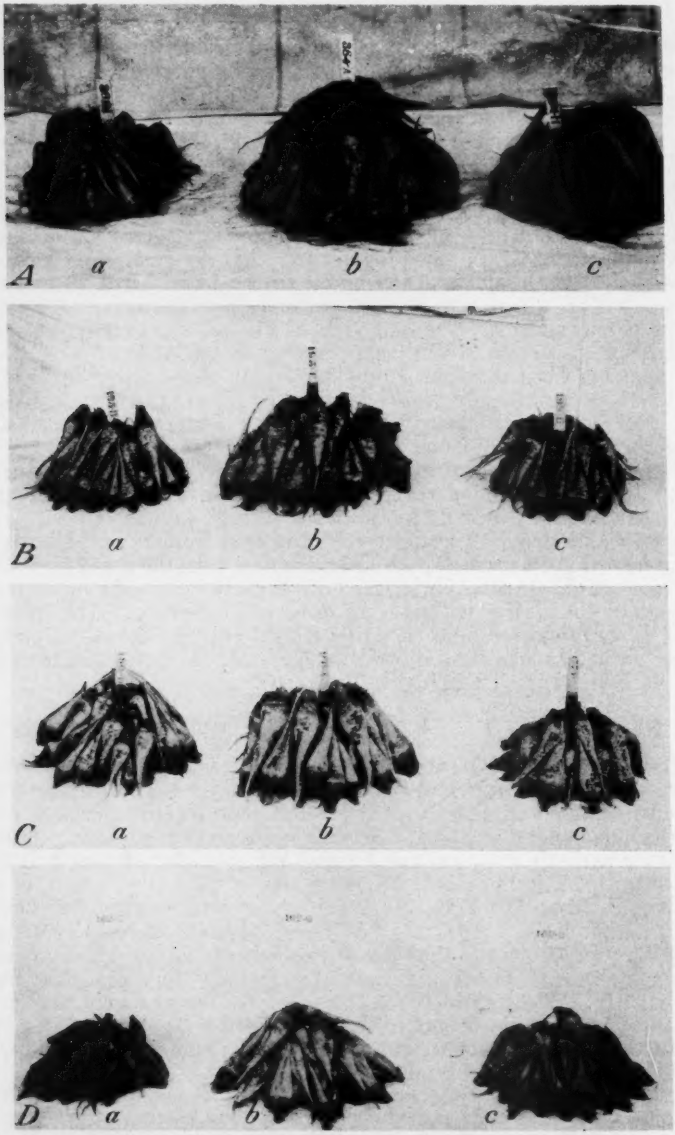

FIGURE 6.—Sugar-beet roots from plots planted with parental strains and with seed obtained by interplanting strains. (Roots taken from border rows but representative of populations from which data were obtained.) *A*, Strain 4a (*a*); (4a×12) (*b*), and strain 12 (*c*). *B*, Strain 7 (*a*), hybrid 8a×7 (*b*), and strain 8a (*c*). *C*, Strain 8b (*a*), hybrid 8b×14b (*b*), and strain 14b (*c*). *D*, Strain 11 (*a*), hybrid 11×Pioneer (17) (*b*), and Pioneer (*c*).

test and other information, have been used in the production of a synthetic variety released for grower use as U. S. 217. A brief report has been made on this variety under the designation Accession 217 (*2*); the strains of which it is composed were referred to as a, b, c, d, and e, corresponding to the numbers 1 to 5 as used here. U. S. 217 has performed satisfactorily in yield and in sucrose percentage, exceeding commercial brands used as checks. It has shown definite leaf spot resistance.

OTHER STRAINS

As shown in table 2, strains 6, 7, 8 (fig. 6, *B* and *C*), and 10 have given some hybrid combinations that show significant gains attributable to hybrid vigor. Strains 14 and 15, which were used only as pollen parents, have given evidence of inducing heterosis response and are discussed in connection with the various strains with which they were combined. Strain 12 was crossed with strains 1 and 4a, the seed not being kept separate as to seed-bearing strain. In the cross with strain 1, the root yield was significantly above the mean yield of the parents. In the cross with strain 4a, significant increase was not shown. Strain 13 was crossed with strain 6, the seed not being kept separate as to seed bearer. The root yield of the hybrid was not significantly above the mean yield of the parents.

Strains 9 (including its substrains a, b, and c), 11, 16, and the commercial brand, Pioneer, were used in crosses and have given additional information on hybrid vigor, to which attention is called.

Strain 9a as mated with strain 1 was extensively used in test 1. The hybrid 1 × 9a, representing the same cross as used in test 1, and the hybrid 1 × 9b and its reciprocal were included in test 2. Because of the close similarity of the substrains, these hybrids are entirely comparable; limited seed quantities, however, made it necessary to omit them from the first test. The root yields as found in test 2 for the hybrid of strain 9 and strain 1 showed for these three cases increases over the mean of the parents of 111, 89, and 84 percent, respectively. If comparisons are based on a mean yield for strain 9, obtained by averaging the root yields of 9a and 9b, this value for strain 9 being in turn averaged with the root yield found for strain 1, the percentages for increase of hybrid over the parental means become, respectively, 201, 199, and 194. In terms of the performance of the commercial brand, the gains in root yield for the three cases are, respectively, 72, 70, and 60 percent. The mean acre yield of roots, 20.19 tons as calculated for the hybrid in test 2, is somewhat less than the average shown in test 1, 22.41 tons; in the two tests, the commercial brand shows comparable differences in its yields. The two tests are in full accord as to the relatively high hybrid vigor shown.

Strain 11, used as a parent in crosses, affords a very interesting series of hybrids for study of heterosis response. The strain is characterized by having a pink epidermis on the root and crown as well as on the basal portions of the petioles, these color characters being dominant. When strain 11 was crossed with strains without pink roots or petioles a definite marker character to identify the F_1 plants was available in the crosses in which strain 11 was the pollen parent. Thus, in the cross 9a × 11, all plants with pink roots or petioles were identified hybrids, and the performance data shown for this cross (test 2, table 2) are based on the F_1 class (fig. 7). The increase in root

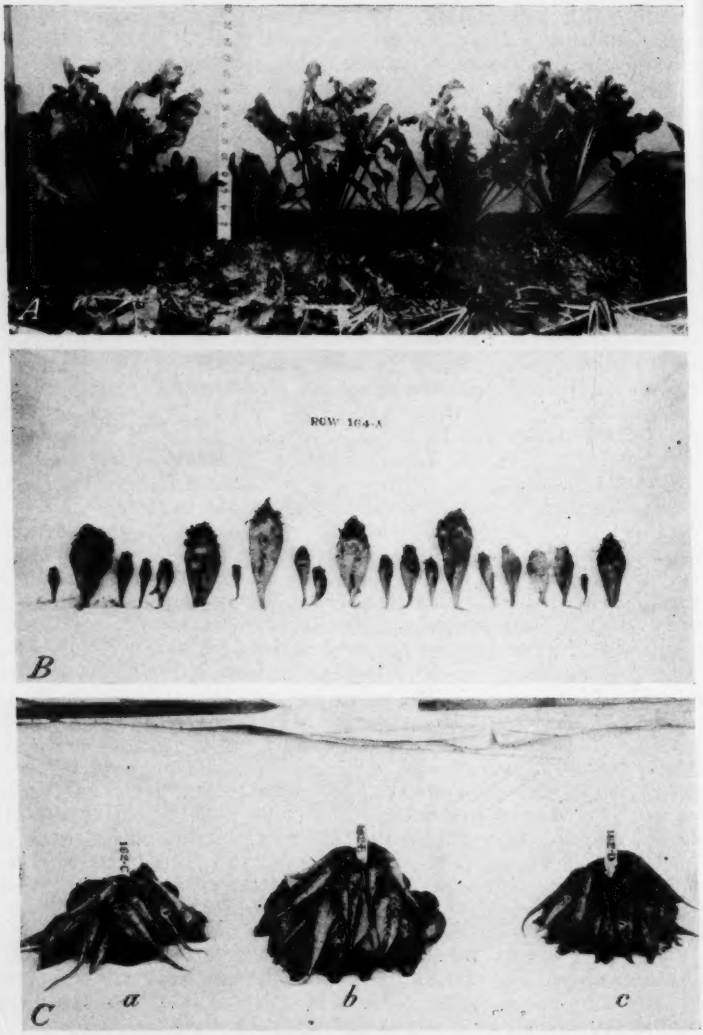

FIGURE 7.—*A*, Hybrid 9a×11. View along the row of a plot planted with seed obtained by interplanting strains 9a and 11, the former being the seed bearer. The ruler stands between two selfs of 9a, the plants adjacent are F_1, their hybrid nature being assured by pink petiole color, a dominant character transmitted by strain 11. *B*, Hybrid 9a×11. Roots from a border row of another plot planted with some of the same seed as the plot shown in *A*. The roots are arranged as they occurred in the row. The five large roots are from F_1 plants, as identified by the pink petiole character. *C*, *a*, Strain 11, as grown in nearby plot. *b*, Hybrid 11×9a. Roots grown in border rows of a plot planted with seed in which strain 11 was seed bearer; roots could not be closely separated into hybrid and selfed classes. These are representative of roots from which data were obtained. *c*, Strain 9a as grown in nearby plot.

yield for the hybrid is more than double that of either parent. In the reciprocal cross, the performance record could not be based entirely on the F_1 class, although the majority of selfs could be eliminated. In spite of this limitation, the performance of the hybrid was outstanding.

The cross 11 × 1 (test 2, table 2) is another example of striking hybrid vigor. It should be noted that strain 11 is very susceptible to cercospora leaf spot. If strain 11 is mated with a resistant strain, for example the cross of strain 11 and strain 1, the hybrid vigor that may be indicated on the usual basis of interpretation is undoubtedly influenced by the unlike effect of the disease on the parental strains. Since resistance to the disease in this cross is at least partially dominant over susceptibility, the hybrid would give relatively a high performance in relation to the more susceptible parent in tests conducted under disease epidemics. It is difficult therefore to assess the extent to which the reaction of disease was a factor in the performance of the crosses of strain 11 with strains 9 or 1. It is certain, however, that the disease factor was not sufficient to account for the striking contrast of hybrid and parents.

Strain 11 as a parent in crosses with strains 1 and 9a had produced hybrids remarkably superior to the parents, yet when crossed with strain 16 it did not produce a similar response. In the cross 16 × 11 (test 2, table 2), the performance of the certified hybrid plants was not appreciably unlike that of the mother strain. Reference to table 1 indicates that strain 16 is removed from the open-pollinated variety by only two generations, one of which was a selfed generation. The heterozygous condition of strain 16 may account for the results obtained.

Selected roots from the commercial brand, Pioneer, were crossed with strain 11 and with strain 1 to produce the type of cross usually designated as top cross. In the cross Pioneer (17) × 11 (table 2), it was possible to base the performance record upon hybrid plants by use of the red-petiole-color character of strain 11, which is not found in Pioneer. In the reciprocal cross, the plot sample could not be reduced so positively to F_1 plants (fig. 6, *D*). In the case of both Pioneer (17) × 11 and the reciprocal, the hybrid yield was about the same as that of Pioneer. The cross Pioneer (17) × 1, and the reciprocal (table 2, tests 1 and 2) show performance records in line with the top cross just discussed.

It may be significant, however, that using inbred strains that are low in yield in top crosses did not result in a hybrid reduced in vigor below the average of the heterozygous variety. Hence, it may be possible by top crossing to impart desirable characters from highly resistant or high-sucrose inbreds to vigorous heterozygous varieties without reduction of yielding capacity taking place.

DISCUSSION AND SUMMARY

The hybrids obtained in more than two-thirds of the crossings reported in these tests have shown significant increase in root weight attributable to vigor of hybridity. With some hybrids no response was shown, and in others the gain found was within chance occurrence. In general, these quantitative effects from hybridization are believed indicative that heterosis is a determinative factor in root yields of sugar beets.

In certain crosses, the hybrid plants were definitely identified by a color character, and in the sampling of plots selfs could be excluded. In one case, the root yield of the hybrid was more than double that of either parental strain. In the cross 1 × 9 and its reciprocal, the determinations were based largely on F_1 plants, since the selfs could be eliminated fairly completely from the plot samples. The hybrid was given extensive test; and it was shown that, regardless of the direction of the cross, the hybrid was significantly greater in root weight than either parent by more than 60 percent. From this, it has been concluded that the effects demonstrated are attributable to heterosis and are not the expression of some simple dominants possessed by one parent. Crosses involving strain 1 as a maternal parent and various other inbreds as pollen parents were consistent in giving hybrids superior in root weight to strain 1. In the crosses in which strain 1 occurred as the pollen parent, the hybrid was often, although not always, superior to the maternal strain. This seemed more likely to occur when strain 1 was mated with vigorous strains.

In the cases in which no attempt was made to separate hybrid and selfed classes in the progeny obtained from a pairing, the performance found was definitely influenced by the presence of lower yielding selfed plants, bringing about a strong trend in the yields toward that of the seed-bearing parent. In spite of this effect of nonexclusion of selfs, many records were obtained in which the progeny from a mating, taken as a whole, was significantly superior to the mean of the parents and frequently superior to each parent entering a cross.

Nearly three-quarters of the hybrid progenies gave root yields 25 percent higher than were found for the commercial brand used in each test. Thus, 7 hybrids out of 10 used in test 2 (table 2), 10 out of 11 in test 3, 6 out of 11 in test 4, and 7 out of 9 in test 5 were significantly superior to Pioneer in root yield. The commercial brand was, in many cases, somewhat more seriously affected by cercospora leaf spot than the more resistant strains or their hybrids. Because of this factor, direct inference as to the effect of hybrid vigor in increasing root weight may not be made, but it seems clear that with certain hybrids the gains in yield over the commercial brand are enough beyond those reasonably attributable to superior disease resistance to indicate definite increase in productivity from heterosis.

Coordinate in importance with the effect of hybridity on root weight is its effect on richness in sucrose. Reciprocal crosses (test 1, table 2) seemed to show a trend in sucrose percentage toward that of the maternal parent. This is believed assignable to incomplete elimination of selfs, since the mean sucrose percentage of reciprocals was approximately equal to the mean sucrose percentage of the parents. In the tests of 41 hybrid progenies, their mean sucrose percentage rather closely approximated the mean sucrose percentage of Pioneer, indicating strong maintenance of requisite quality.

It is customary to evaluate sugar-beet varieties in terms of gross sugar produced per unit of area. Because of the definite indications for many of the hybrids of strikingly increased weights over the parental material and of sucrose percentages about equal to the mean of parents, the increase in productivity in terms of sugar follows essentially the response in root weight attributable to hybrid vigor.

The situation as to the yielding capacity of inbred strains is in a measure shown by these tests. Among the 16 strains used, some of

which are the product of many generations of inbreeding, 2 were found, under the conditions of these experiments, to be significantly superior and 2 significantly inferior to the commercial brand in acre yield of roots. The other strains did not differ significantly in root yield from Pioneer. The average performance of the inbred strains and that of the commercial brand were very similar. Certain strains were strikingly resistant to cercospora leaf spot, while others were susceptible. As a group, they were more resistant than the commercial brand, a factor which must be taken into account in consideration of relative yielding capacity. The performance of many of the inbred strains as they now exist warrants the opinion that their productivity is still relatively high after a number of generations of inbreeding.

The experience with first-generation hybrids suggests that in the program for sugar-beet improvement purposeful crossing of strains found to give strong heterosis response may have an important place. Introduction of a synthetic variety, U. S. 217, produced by intercrossing five of the strains which were found in these tests to give indications of such response and which were, in addition, high in cercospora leaf spot resistance, represents an attempt in this direction.

The practical breeding problems to be faced in production of high-yielding hybrids or desirable synthetic varieties hinge in part upon the production of disease-resistant and otherwise desirable inbred strains. Advantageous combinations probably will need to be found because of the general tendency of yielding capacity to decrease with inbreeding. Seed setting among the strains has not been excessively low, whence it would appear that seed stocks of these strains in considerable size may be produced without excessive cost. The important problem of securing a high degree of intercrossing between chosen strains must be faced. The only practical method now available of securing intercrossing seems to be to pool the seed of strains for planting the seed field. In the production of U. S. 217, the five inbred strains were variously paired to produce stock seed in three fields, and the stocks thus obtained were pooled for use as planting stock for commercial seed production by the field-overwintering method. A study of the synthetic variety thus produced indicates that a fairly high degree of intercrossing took place, but many apparent selfs were found. Observations made in the course of these tests with single crosses showed that the amount of cross fertilization occurring may vary from as much as 90 percent to less than 10 percent. Further, it is known that climatic conditions affect the rate of seed-stalk formation, seed setting, and viability of strains differentially, making it necessary that response of strains to these factors be taken into account in the sugar-beet-breeding program.

Certain crosses also indicate that heterozygous varieties otherwise desirable may be improved with respect to specific characters by the method of top crossing. By a course of intensive inbreeding and selection in segregating populations, it is possible to make faster progress in establishment of characters such as high disease resistance or high sucrose percentage than will be accomplished by mass selection. In the event that reduction in vigor takes place, it appears from these tests that the inbred strains which can contribute a desired character may be used in top crosses without necessarily reducing the growth potentialities of the heterozygous mother.

Methods employed by European firms engaged in production of sugar beet seed are essentially mass-selection methods. A brand or variety as supplied consists of a heterozygous population which, as a result of adherence to progeny tests in the selection of breeding stock, conforms in average performance with reasonable fidelity to a given physiological classification, such as tonnage, intermediate, or sugar type. As might be expected, improvement has been slow. That there has been increase in productivity in recent decades has been questioned (*3*). Varieties with special adaptation have, in general, not been produced. In many respects, the European varieties of sugar beet are comparable in status to high-grade open-pollinated corn varieties. The evidence that strains of sugar beet can be produced which have special characteristics such as disease resistance or high sucrose percentage and the showing that definite heterosis response exists between certain strains opens up definite opportunities in development of varieties better adapted for American conditions and actual increase in sugar beet productivity.

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ROOT RESPONSES OF NONINFECTIOUS HAIRY ROOT APPLE SEEDLINGS UNDER DIFFERENT METHODS OF PROPAGATION[1]

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INTRODUCTION

In the last decade the commercial production of apple (*Malus*) seedlings for use as rootstocks has greatly increased in the region around Yakima, Wash. During this period seedling growers, especially in this region, have been using in large amounts domestic American seed obtained from various commercial apple varieties of or the Northwest.

In cooperative experiments with several nurserymen, the writers have had an opportunity to compare seedlings grown from imported French crab with those grown from domestic seed. It has been observed repeatedly that a relatively large percentage of the seedlings from domestic seed are characterized by a condition on their roots that has been termed "hairy root" (*28*)[2] and "simple form hairy root" (*11, 17*). The latter term was used to describe 1-year-old seedlings that exhibited excessive lateral roots in the region extending from the collar to a distance of several inches below the ground line.

In conversation and correspondence with nurserymen the writers have referred to this type of malformation as "noninfectious hairy root," because no evidence that the condition is the result of infection has been noted either in the literature or in field observations.

The situation resulting from the use of domestic seed has created a problem of considerable economic importance to the seedling grower and to the commercial propagator. Field counts indicate that approximately 20 percent of the average run of domestic seedlings are affected. The subject is also of interest to the investigator concerned with the problem of rootstocks, because many of the clones of the Malling types of Paradise and other dwarfing apple stocks as selected by Hatton (*5, 6*) exhibit root systems that show conditions somewhat similar to noninfectious hairy root. The extent of this similarity, however, cannot be determined until comparative observations are made on 1-year-old plants of these Malling clones.

The studies reported herein were undertaken to obtain information on the nature of the root systems of these abnormal seedlings and their responses under different methods of propagation. Specifically information was needed as to what advice should be given to nurserymen regarding the use of normal-appearing portions of affected seedlings.

[1] Received for publication February 7, 1940.
[2] Italic numbers in parentheses refer to Literature Cited, p. 753.

REVIEW OF LITERATURE

In Downing's time (*4*), the fact that root systems differed, especially in the effect on the scion variety, was well recognized. He noted the dwarfing effect produced on apple and cherry when worked on Paradise and "perfumed" (mahaleb) roots, respectively. Paradise and Doucin stocks were imported into this country for many years specifically for producing dwarf trees (*18*, *35*). In general, these stocks were characterized by aerial burrknots and by masses of roots growing in tufts or clusters near the ground line. Their rooting habits and tendency to produce shoots near the ground line rendered them especially adaptable to propagation by "stooling."

Knight (*13*) and many others have referred to the use of burrknots as an aid to rooting. The practical synonymy of the terms "burrknots," "aerial crown gall," "stem tumors," etc., has been noted (*17*, *27*, *30*), and despite the fact that symptoms have been produced by inoculations (*27*), the evidence indicates that these malformations are not caused by a pathogenic organism but are of a genetic nature (*9*, *10*, *16*, *17*, *20*, *29*). That individual plants exhibiting burrknots are characterized generally, if not invariably, by abnormal rooting is indicated by the illustrations of several investigators (*5*, *6*, *11*, *17*, *22*) and by general observations.

The term "hairy root" (*28*) was used to describe the large masses of roots on the main root axes of nursery apple trees. Later, Hedgcock (*11*) used the term "simple form hairy root" for these and similar malformations on the stem and roots of apple seedlings. He described the symptoms as consisting "of numerous small roots growing out at nearly right angles, either singly or in tufts, from an older root or stem." In the absence of information as to the cause of this condition, he naturally advocated segregation of affected seedlings as a routine precautionary measure. However, it is significant that he concluded (1) that the condition was not associated with wounding, (2) that it was practically eliminated when affected seedlings were not used in grafting, and (3) that when affected seedlings were used in piece-root grafting there followed in the second year "an atrophy of the hairy root pieces."

As a result of experiments in which apple seeds were grown in steamed soil, Muncie (*21*) concluded that "the fibrous type of hairy root is probably not infectious" and that the condition is the result of some inherent character of the seedling. Later, however, Muncie and Suit (*22*) showed a seedling with the "fibrous type of hairy root" as "characteristic of the condition found on nursery trees grown in sandy soil." They stated that on the quince the formation of clusters of roots appears to be the natural rooting habit and is not associated with bacterial infection. Maney (*17*) planted several hundred 1-year-old affected apple seedlings and noted that the masses of rootlets that subsequently originated on the stem arose "from typical burrknots." He concluded that "the simple form of hairy root on apple seedling stock is caused by burrknot."

The problem of adequately describing various types of root systems is obviously a difficult one. That individual roots and root systems as a whole or in part may exhibit quantitative and qualitative differences is well recognized (*5*, *8*, *14*, *23*, *24*, *26*, *32*, *33*). Criteria

for descriptions, however, are wanting (*31*), and as a result considerable confusion exists. The various interpretations that have been placed on the word "fiber" illustrate this point. Other difficulties involved include variations due to the many environmental factors that may be encountered and to the fact that different parts of the root system often display unlike characteristics.

Under the existing situation, therefore, emphasis in this report will be placed on quantitative features and, as a matter of expediency, root systems that exhibit a comparatively large number of small lateral roots on the current season's growth will be designated as abnormal (affected). Although there is no sharp dividing line between the normal and abnormal, nevertheless symptoms of abnormality as they refer to excessive numbers of lateral roots originating on a well-defined axis, can be roughly classified according to degree, as slight, moderate, or pronounced.

MATERIALS AND METHODS

The experiments were performed with the use of (1) root cuttings, from which plants were propagated directly; (2) grafts, made from piece-root cuttings; (3) 1-year-old seedlings; and (4) seed from various sources. Plantings were made either in steamed soil in the greenhouse or in nontreated soil in the field.

Seedlings showing abnormalities in roots or in rooting are designated "noninfectious hairy root seedlings," and the symptoms are classified as slight, moderate, or pronounced, on a quantitative (numerical) basis. The parts of these affected seedlings not exhibiting symptoms are referred to as "clean" to distinguish them from similar-appearing areas on normal plants.

EXPERIMENTS AND RESULTS

PRELIMINARY EXPERIMENTS

Because of the difficulty of classifying seedlings that exhibited very slight symptoms of noninfectious hairy root, several hundred 1-year-old seedlings that were classed as "borderline" cases were planted in 1920 to observe their future development. When dug 1 year later, approximately 50 percent of these seedlings showed definite symptoms. This experience was of value in subsequent experiments, since it afforded some basis for diagnosis in discarding seedlings intended for use in crown gall and hairy root experiments.

In preliminary experiments in 1936 a small number of root cuttings were made from the seedling (domestic) roots of several 1-year-old grafts that showed moderate symptoms of noninfectious hairy root. The cuttings were planted horizontally about 1 cm. deep in sterile soil in a greenhouse and grown in comparison with root cuttings obtained from grafts of a clonal material known as U. S. D. A. Vt. 323, which had been under observation for several years and which had never shown symptoms of abnormal rooting. When examined 1 year later the U. S. D. A. Vt. 323 root cuttings were normal; the six surviving plants of the moderate noninfectious hairy root series showed relatively slight symptoms on the original root piece, but pronounced symptoms at the base of the new shoot growth.

In the same year, root cuttings from the "clean"-appearing areas of noninfectious hairy root seedlings were planted. All of the 12 surviving plants showed symptoms.

The results of these preliminary experiments with root cuttings indicated that although the condition is systemic or inherent, stem rooting tends to reduce the severity of the symptoms on the original root piece. Accordingly, the experiments of 1938, reported subsequently, were designed to furnish additional evidence along these lines.

Included in these earlier experiments is a report of the results obtained in comparisons of seedlings grown from seed procured from various sources. Field observations had indicated that the progeny of domestic seed were particularly affected. The data in table 1 show wide variations in the percentage of noninfectious hairy root seedlings in the progeny of certain crosses and in the seedlings from certain seed sources.

TABLE 1.—*Amount of noninfectious hairy root observed on 1-year-old seedlings segregated as to source of seed*

Year	Source of seed	Locality where grown	Seedlings examined	Noninfectious hairy root seedlings
			Number	*Percent*
1936	Domestic	Beltsville, Md. (steamed soil, greenhouse).	54	20
	French crab	do	49	2
1937	Domestic	Beltsville, Md. (field)	520	16
	French crab	do	322	4
	Domestic	Kansas	300	16
	French crab	do	300	2
	Delicious	Beltsville, Md. (field)	200	2
	Rome Beauty × O[1]	do	200	5
	Winesap × O	do	200	5
	French crab	do	200	0.5
1938	Baldwin × O	Beltsville, Md.	35	0
	Ben Davis × O	do	350	16
	Bonum × O	do	147	21
	Delicious × O	do	244	5
	Domestic × O	do	50	16
	Gallia × O	do	204	14
	Grimes Golden × O	do	87	13
	Golden Delicious × O	do	220	18
	Jonathan × O	do	136	0
	King David × O	do	70	0
	Macoun × O	do	41	22
	McIntosh × O	do	170	13
	McIntosh × Delicious	do	151	30
	McIntosh × Northern Spy	do	219	24
	Nero × O	do	100	0
	Northern Spy × O	do	342	32
	Northwestern Greening × O	do	220	9
	Oldenburg (Duchess) × O	do	63	0
	Rome Beauty × O	do	225	15
	Stayman Winesap × O	do	53	5
	Starking × O	do	164	4
	Stella × O	do	105	2
	Summer Pearmain × O	do	102	12
	Transcendent × O	do	20	0
	Wealthy × O	do	100	0
	Williams × O	do	165	0
	Winesap × O	do	108	17
	Winter Banana × O	do	299	9
	Yellow Transparent × O	do	35	0
	York Imperial × O	do	191	22

[1] "× O" signifies open or unprotected pollination.

EXPERIMENTS WITH GRAFTS IN 1938

Forty-three seedlings showing symptoms of noninfectious hairy root ranging from moderate to pronounced were selected, and each

seedling was given an individual number and thus identified as clonal material. These seedlings showed excessive rooting, extending from the hypocotyledonary region at the ground line to a distance ranging from 2 to 4 inches down the main axis. Typical examples are shown in figure 1, *A*, *B*, *C*, in comparison with a normal seedling (*D*). The

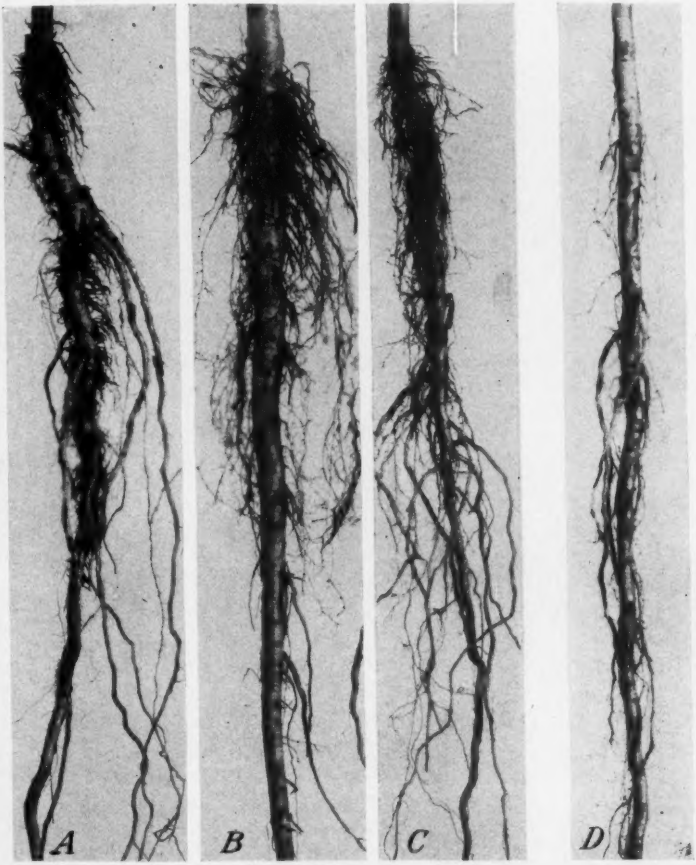

FIGURE 1.—One-year-old apple seedlings typical of those used in the experiments: *A*, *B*, Seedlings showing moderate symptoms of noninfectious hairy root; *C*, seedling showing somewhat less symptoms; *D*, normal seedling.

lower portions of the main axis and most of the side roots of these noninfectious hairy root seedlings appeared normal in all respects; such normal-appearing areas are designated "clean."

Stem cuttings (scions) were grafted to the proximal root pieces of the seedling from which they were cut, thus preserving the original combination of each graft. Of these 43 grafts that were planted in the

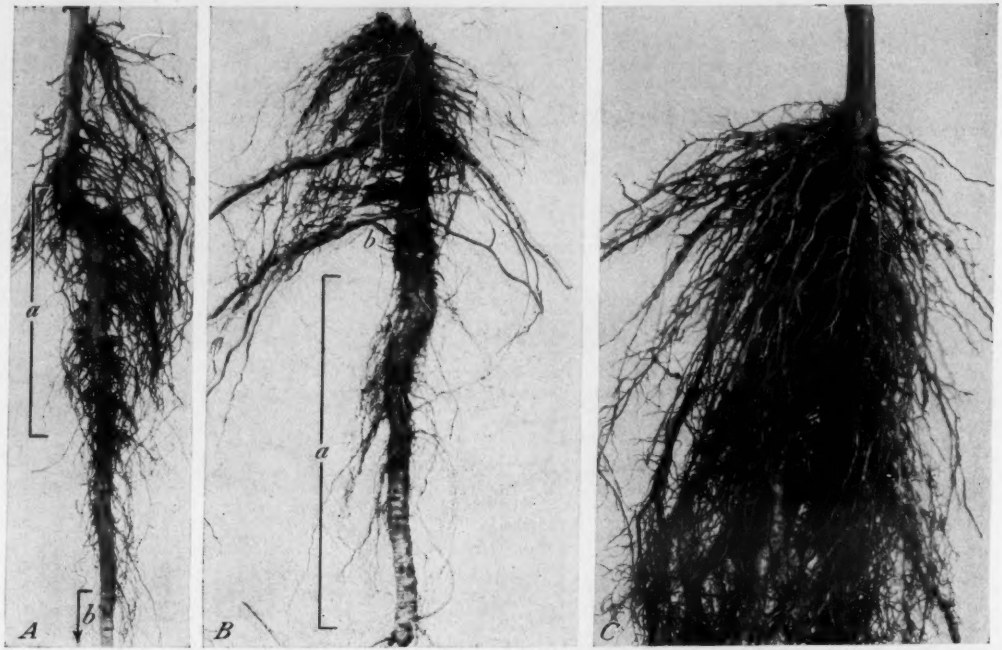

FIGURE 2.—Results obtained in the grafting and root-cutting experiments. *A*, The original 1-year-old seedling shows moderate symptoms. The region designated at *a* was used in the piece-root grafting to a scion taken from the stem of this seedling. The "clean" area, *b*, at the distal end of this seedling, was used in the root-cutting experiment. *B*, 1-year-old tree resulting from grafting a scion from the seedling shown in *A* to the root piece shown in *A* at *a*. The scion has rooted and the symptoms on the original root piece *a* have been suppressed. The union is at *b*. *C*, 1-year-old tree resulting from growth of root cutting made from the "clean" (distal) region of seedling shown in *A*, *a*. There is no stem rooting, and the symptoms on the root are pronounced.

field, 40 grew and were examined as 1-year-old trees. The results (table 2) show that the symptoms on the original root pieces of the 1-year-old grafts were in all cases less pronounced than when they were originally grafted, as judged by comparison with photographic records. In all cases slight to pronounced rooting occurred on the scion; the more pronounced the scion rooting, the more the symptoms were suppressed on the original root piece. As an example, the condi-

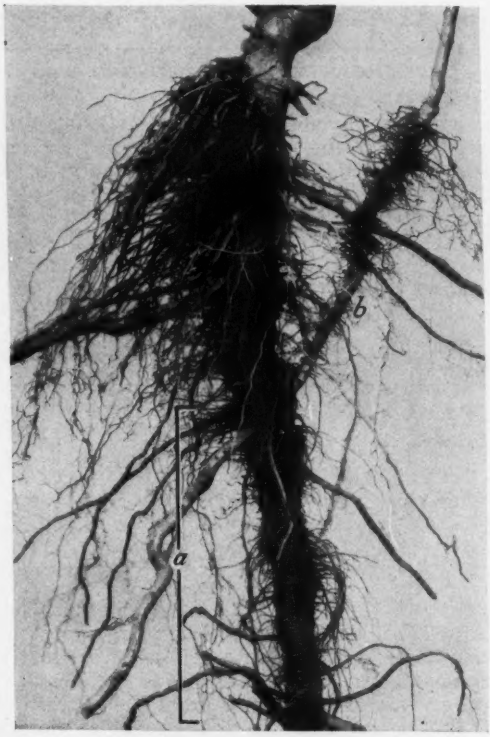

FIGURE 3.—A 1-year-old graft resulting from grafting a scion, from a seedling showing pronounced symptoms, to its own root piece. Pronounced symptoms are apparent on the scion part of the graft, but the symptoms on the original root piece (*a*) have been suppressed. The side shoot (*b*), originating just below the graft union, exhibits pronounced symptoms at the region near the ground line.

tion of the root in the original seedling is shown in figure 2, *A;* in the 1-year graft in *B;* in the plant that grew from a root cutting from a "clean" portion near the distal end of this seedling in *C.* In this illustration the symptoms on the original seedling now incorporated in the 1-year graft are classed as slight. As might be expected, the underground parts of adventive shoots that occasionally sprouted from below the graft union and subsequently rooted exhibited pronounced symptoms at the distal regions (fig. 3).

TABLE 2.—*Results obtained on grafts whose root pieces showed moderate to pronounced symptoms of noninfectious hairy root when grafted*

Symptoms on original root pieces of 1-year-old seedlings	Grafts that grew	Symptoms on the 1-year-old trees					
		On original root piece			On scion		
		Slight	Moderate	Pronounced	Slight	Moderate	Pronounced
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Moderate	21	21	0	0	6	12	3
Pronounced	19	0	19	0	5	9	5

EXPERIMENTS WITH ROOT CUTTINGS IN 1938

Seventy-eight piece-root cuttings were taken from the same 43 noninfectious hairy root seedlings the proximal root pieces of which were used for grafting in the preceding experiment. These root cutings were classified either as "clean" or with respect to the degree of symptoms displayed. The cuttings were segregated according to the seedlings from which they were obtained. They were planted vertically in pots in steamed soil with the top ends partly above ground so as to prevent rooting from the new shoots. Forty-four of these root cuttings produced plants that were dug as 1-year-old trees and classified as outlined in table 3.

TABLE 3.—*Results obtained from planting root cuttings taken from noninfectious hairy root seedlings*

Symptoms on root cutting	Cuttings that grew	Symptoms on the 1-year-old plants			
		Slight	Moderate	Pronounced	Moderate to pronounced
	Number	*Number*	*Number*	*Number*	*Number*
"Clean" (no symptoms)	32	2			30
Slight	2	0	2	0	
Moderate	8	0	6	2	
Pronounced	2	0	0	2	

The results show that under suitable conditions the "clean" (symptomless) parts of affected seedlings will develop symptoms. In the absence of scion rooting, the symptoms on the original root pieces either were made manifest on plants from the 32 "clean" cuttings, or were in general accentuated on plants from the affected cuttings, as determined by comparison with the photographs of the original roots (fig. 2, *A*, *b*; *C*).

For comparison, 50 seedlings that appeared normal were selected for root-grafting and root-cutting experiments. A piece of the stem from each seedling was grafted on the proximal part of its own root. When dug 1 year later the root systems of 47 of the grafted trees were normal; the remaining 3 showed moderate symptoms of noninfectious hairy root on the original root piece. In the lot of 47 trees, scion rooting invariably occurred; but these scion roots generally occurred singly, and from their size and texture they were tentatively considered as normal (fig. 4, *A*).

Of the 100 root cuttings from the 50 seedlings selected as normal, 56 grew. The root systems of 53 of the resulting 1-year-old plants were classed as normal (fig. 4, *B*); 3 exhibited moderate symptoms of noninfectious hairy root.

The fact that a few of the plants grown from parts of normal seedlings evidenced symptoms of noninfectious hairy root was somewhat surprising. The percentage is considerably higher than that usually encountered in routine grafting experiments made by the writers.

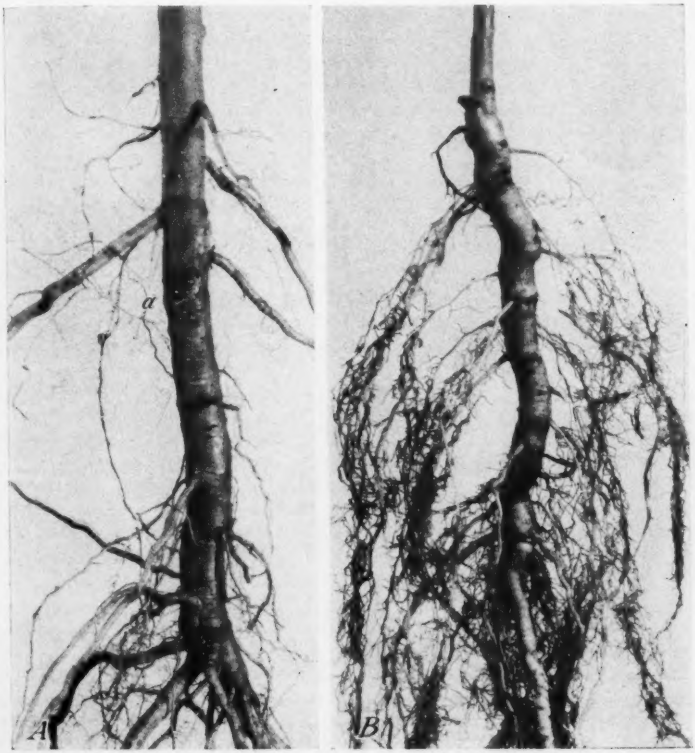

FIGURE 4.—Trees resulting from the use of normal seedlings. *A*, A 1-year-old graft resulting from grafting a scion from the seedling to its own root piece. The lower part of the graft union is at *a*. Despite the scion roots, which are considered normal, the caliper of the root piece is very good. *B*, A 1-year-old tree resulting from a piece-root cutting taken from a normal seedling. Note the branching habits of the numerous smaller roots.

It is possible that rooting above the collar may have masked noninfectious hairy root symptoms on these seedlings when they were selected.

EXPERIMENTS WITH 1-YEAR-OLD SEEDLINGS

In addition to the experiments with grafts and root cuttings, twenty-five 1-year-old seedlings showing moderate symptoms were cut back to approximately 10 inches of stem growth and were planted horizontally to encourage rooting from the buried stem tissue. Of the 23 plants surviving 1 year later, 21 had rooted from the stem, and the noninfectious hairy root symptoms on the original root were suppressed in direct proportion to the amount of stem rooting. These stem roots all came from the regions of the buds, usually in clusters of several to

many. The plants shown in figure 5 were selected to illustrate various types of stem rooting and to show the lack of symptoms on the original roots, which had exhibited moderate symptoms when planted. The plants shown in figure 5, *A* and *B*, were typical of the lot; that shown

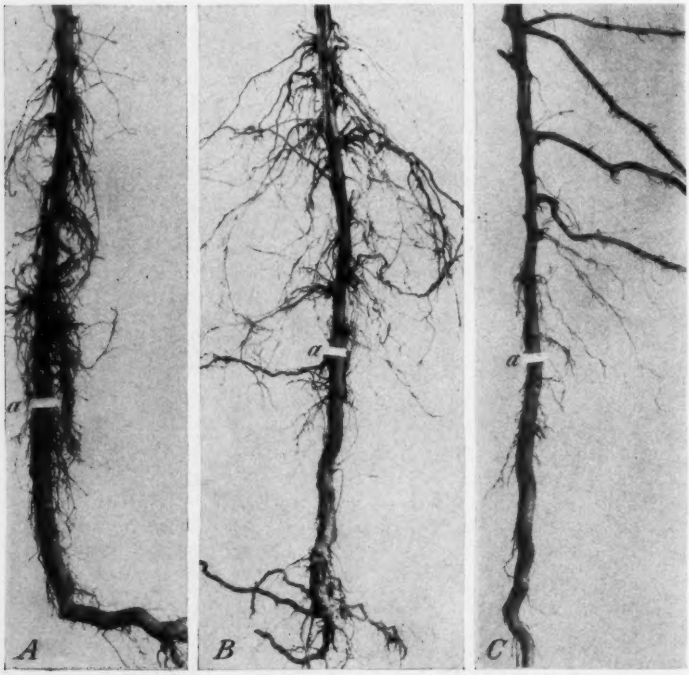

FIGURE 5.—Two-year-old seedlings from 1-year-old layered affected seedlings. Various types of stem rooting are shown, and the symptoms on the original roots have been suppressed. The original root-stem junctions are at *a*. *A* and *B*, More typical examples of the types of stem rooting encountered. *C*, Stem rooting is practically normal in appearance, but there were slight indications of symptoms on some of these roots.

in *C* exhibits what may be tentatively considered normal stem rooting in mode, number, and texture. However, a slight tendency to exhibit noninfectious hairy root symptoms is observed on some of these stem roots. Considerable variation has been observed in the type and number of roots arising on stem tissue, even on the same plant. The 2 plants that did not push stem roots made very poor growth and the symptoms on the original root pieces were pronounced.

DISCUSSION

The reasons for using the term "noninfectious hairy root" have been given. Although the possibility that a virus may be an etiological factor should not be disregarded, there is at the present time no evidence that the condition is infectious. Hedgcock *(11)* concluded that the condition was not associated with wounding, in contrast with other types of malformations that he described as "soft crown gall,"

"hard crown gall," and "woolly knot" form of hairy root. Muncie's *(21)* experiments in which he grew apple seed in steamed soil furnished better evidence that the condition is not caused by a pathogen. Other evidence favoring this point of view includes (1) the fact that symptoms have never been observed on the current season's growth of root systems when the older roots are normal; (2) the lack of spread of symptoms to scion part of graft on commercial varieties; (3) the failure of symptoms ever to appear on certain clones that consistently have exhibited normal roots; and (4) the ability either to suppress or to produce the symptoms at will, as demonstrated in these experiments.

The experiments in growing grafts and root cuttings were designed to afford a strict comparison of the reactions of root cuttings with those of grafts, clone for clone. These experiments also furnished a close comparison of the reactions of 1-year-old seedlings with 1-year-old grafts. The regrafting of the stem part of a seedling to its own roots merely simulated the commercial propagation of different varieties by grafting, but it was reasoned that this procedure would afford an opportunity for scion rooting with the elimination of complicating factors due to either mechanical or physiological incompatibilities.

The results of the experiments of 1938 confirm those of previous years and demonstrate that this noninfectious hairy root condition is inherent and that the symptoms are influenced by the degree of stem rooting. The grafts were planted in a normal manner and thus were afforded an opportunity for scion rooting; the root cuttings were planted so as to eliminate rooting from the newly pushed shoots. All of the grafts pushed roots from the stem portion, presumably because of the juvenile condition of the scion wood, and the symptoms of noninfectious hairy root on the original root piece were less marked than they were at the time the grafts were made. However, in the case of the root cuttings the newly pushed shoots did not bear roots and the symptoms were either made manifest or were intensified on the original root piece, depending upon whether it came from a "clean" area or from an area exhibiting symptoms. It follows that the use of any portions of noninfectious hairy root seedlings in propagation will result in manifestations of the symptoms unless the scion variety pushes vigorous scion roots.

The experiments on grafts and on the 1-year-old seedlings are closely comparable. By planting the seedlings horizontally (layering), the stem portions were afforded an opportunity for rooting, and the results are similar to those of the grafting experiment in that stem rooting suppressed the symptoms on the seedling roots.

The experiments with grafts and root cuttings made from normal roots demonstrated that in general the root systems remained characteristic for normal plants. That errors of judgments, however, may be made in classifying seedlings is to be expected, in view of the fact that symptoms are so varied. The fact that over a period of years no symptoms have ever been observed in the thousands of 1-year-old plants grown from root cuttings of U. S. D. A. Vt. 323 is an indication of the constancy of characteristics of root systems, on young plants at least.

For convenience, and in the absence of definite standards for a normal root system, noninfectious hairy root symptoms on 1-year-old seedlings were classified on a quantitative basis as slight, moderate, or pronounced. An excessive number of lateral roots is the chief dis-

tinguishing characteristic, regardless of their morphological arrangement on the main axis. These roots may be either (1) in closely spaced fascicles (burrknot type), (2) in a more or less linear arrangement, or (3) scattered promiscuously around the axis, as illustrated in figure 1, *A*, *B*, and *C*, respectively. Obviously, intergradations occur between all of these arbitrarily selected and somewhat indefinite "types," as well as between normal and affected seedlings. Although these roots generally have a relatively thick phloem region ("cortex"), this more or less "fleshy" condition is apparently usual for abnormally developed roots and presumably is caused by improper functioning. A somewhat distinguishing characteristic is the lack of branching of the smaller roots as compared with normal feeding roots. And, finally, it should be noted that normal-appearing roots may be interspersed along the axis with these abnormal roots.

Two subjects of interest in connection with root systems that display noninfectious hairy root symptoms are (1) the degree of association with aerial burrknots, and (2) the possible dwarfing effect when used as rootstocks. In the absence of evidence obtained in a comparison of clones selected for the purpose of determining these points and propagated by the same methods, a detailed discussion of these questions is not warranted. In general, however, clones that display burrknots are recognized as being good stem rooters (*6*, *7*, *10*, *17*), and the tufts of roots arising from burrknots may be considered as an abnormal condition similar to noninfectious hairy root (*17*). It has been observed (*10*) that although not all Paradise (layered) stocks produce aerial knots, they do develop "tufts of roots resembling those that grow from burr knots."

Unfortunately, there is no experimental evidence on the question as to whether seedlings that exhibit noninfectious hairy root symptoms will, when mature trees, develop root systems similar to those of the dwarfing Malling types of Paradise and other dwarfing rootstocks. The root systems of severely affected 1- and 2-year plants are characterized by regions in which small lateral roots occur in such profusion as to result, presumably, in a restriction of normal functioning. Further, the parts of the root situated distally to (below) regions displaying pronounced symptoms frequently are considerably atrophied and bare of small lateral feeding roots. Thus the root systems of affected plants should eventually become limited in size in the sense that the quantity of lateral roots bearing the finely divided feeding roots will be relatively small.

That a limited size of the root system is an important factor in dwarfing is a general conclusion justified by the evidence submitted by several investigators (*12*, *25*, *26*). It is therefore suggested that the responses obtained in the affected plants in these experiments may offer a reasonable explanation for dwarfing on the basis that the number of roots that can function efficiently is relatively small.

These studies indicate that in affected young plants the expression or manifestation of symptoms may be largely dependent upon the influence or dominance of some proximal region. To further illustrate this dominance, and to indicate that the degree of atrophy is greater than is usually encountered, reference is made to Lincoln (*15*, *fig. 1*, *No. 4*) and to Hedgcock (*11*, *pl. VII*, *fig. 1*).

That some characteristics may change as the root system becomes older is indicated by descriptions of the dwarfing clone Malling type

IX, which as a 4-year-old tree may exhibit "much fibre chiefly on the upper part of the root system" (*34*), but later (*2*) as a 15-year-old tree, on which, however, Bramley's seedling had been grafted, there is no evidence of "fibre," and the long lateral roots bear comparatively few normal finely divided feeding roots.

The above discussion is an attempt to envision the question of dwarfing from a more or less quantitative standpoint. It is desired to emphasize, however, that qualitative factors are undoubtedly of great importance. The anatomical studies on dwarfing stock by Beakbane and Thompson (*3*) and the studies by Rogers (*25*) on root growth are of fundamental importance. These workers have given a reasonable explanation for dwarfing, particularly from the standpoint of anatomy (*3*), and an attempt has been made to establish the idea of root nomenclature as an aid in description (*25*).

Many factors, such as environment, physiological reactions, age, and nutritional and edaphic conditions, undoubtedly affect the appearance of a root system; but the conclusions of others (*24, 34*) that most clones have identifying characteristics indicate that the inherent qualities are frequently of decisive importance (*1, 2, 6, 12, 33*). In order to observe these inherent qualities, it is highly desirable to use methods in propagation and in culture that will result in an optimal expression of growth potentialities in 1- and 2-year-old plants. When these conditions obtain, some differentiation of the factors that may subsequently affect the root systems (*1*) may be more readily afforded. Tydeman (*32*) noted the difficulty in classifying "the adventitious systems evolved on" young shoots of stooled seedlings. The present experiments suggest the desirability of using 1- and 2-year-old plants grown from root cuttings as a basis for determining root characteristics of clonal stocks.

The more immediate practical considerations involved in these investigations are concerned mainly with the question of malformations on apple nursery stock. Hedgcock's (*11*) illustrations and comments on seedlings exhibiting these symptoms of noninfectious hairy root served to warn the writers that this disorder was probably noninfectious, and accordingly his recommendations to discard suspected seedlings for propagation purposes were followed by the writers in experiments on control of crowngall and hairy root. As a result, although in the past nonpathogenic hairy root may have been confused with pathogenic hairy root (*19*), this disorder has been practically eliminated in all of the writers' experiments since their inception. In the grafting experiments during the last 5 years, however, the writers have noted an occasional noninfectious hairy root tree, particularly in trees grown from domestic seedlings. The results of the present experiments indicate that symptoms may be somewhat masked on a small percentage of 1-year-old seedlings.

In order to eliminate large losses due to culling, seedling growers should give consideration to the fact that the number of seedlings showing this disorder is much larger in the progeny of many of our commercial apple varieties than in that of the French crab. The data in these experiments, supported by observations on general growth habits, however, indicate that seedlings of the Delicious variety are suitable to use (*35*). Relatively early germination and exceptionally good stands have been obtained from seed of this variety in several

tests. The Delicious variety itself makes exceptionally good root-graft unions, and its seedlings are comparatively uniform.

Although in this discussion emphasis has been placed on the fact that certain rather localized characteristics of a root system can be changed, it should be equally emphasized that the general character of the root system remains constant when conditions favoring vigorous growth are made available. The fact that root systems of vegetatively propagated clones possess distinguishing characteristics is well recognized. Thus, Malling types have been differentiated (*5*), and Lincoln (*14*) has illustrated root systems that he considers characteristic of many of our commercial varieties. The latter's illustration of Northern Spy roots appears to show pronounced symptoms of noninfectious hairy root. This variety, in common with the Malling dwarfing clones, is rather readily propagated by layering and is considered a semidwarfing stock (*1*).

CONCLUSIONS AND SUMMARY

Experiments in which apple seeds from known sources were grown have confirmed field observations that relatively large percentages of 1-year-old seedlings from commercial (domestic) varieties exhibit pronounced symptoms of the disorder formerly known as "simple form hairy root." It is proposed to call this disorder "noninfectious hairy root" in order to emphasize the fact that no pathogenic organism is associated with the condition, which is characterized by an excessive number of lateral roots on the main root axis.

As a result of comparisons of seedlings from various seed sources, including French crab, it is evident that relatively large percentages of seedlings exhibiting these symptoms of this disorder are found in the progeny of many commercial (domestic) varieties. The Delicious variety, however, is an exception, in that a comparatively small percentage of its seedlings are affected.

In order to observe the reaction of affected seedlings under different conditions of growth and to demonstrate the inherent or genetic nature of this disorder, experiments were performed with the use of grafts, root cuttings, and 1-year-old seedlings. Scions from 1-year-old seedlings were grafted to the proximal portions of their own roots; root cuttings were made from the affected portions of these same seedlings and also from regions that appeared normal. In order to preserve the identity of the resulting plants, each original seedling was numbered and treated as clonal material.

The conditions of growth were varied mainly in respect to scion rooting. The use of scion wood from these "juvenile" plants practically insured scion rooting on the grafted plants; by planting the proximal ends of the root cuttings above ground level, scion rooting was precluded.

An examination made 1 year after planting revealed the following conditions:

(1) On the grafts, scion rooting occurred in all cases, and the symptoms on the original root piece were suppressed, generally in direct proportion to the amount of scion rooting.

(2) On the 1-year-old seedlings (layered), stem rooting occurred in practically all cases, and the symptoms on the original root pieces were suppressed in agreement with the results obtained on the grafts.

(3) On the plants grown from root cuttings that had exhibited symptoms when planted, no stem rooting occurred and the symptoms

on the original root piece were intensified. On the plants grown from root cuttings from "clean" (symptomless) regions of affected seedlings, symptoms were manifested.

Because it is the common practice for nurserymen to discard all trees that exhibit symptoms of noninfectious hairy root, it follows that, in order to avoid loss through discarding, no part of affected seedlings should be used for the usual propagation purposes.

Although it is not assumed that all clones that exhibit symptoms will cause dwarfing, it is suggested that selections from clones that exhibit the more pronounced symptoms may readily furnish sources for dwarfing rootstocks.

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THE EFFECT OF CONTROLLED CULLING OF CHICKENS ON THE EFFICIENCY OF PROGENY TESTS[1]

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INTRODUCTION

A complete program of progeny testing as visualized today involves compilation and analysis of extensive records on a representative sample of the progeny of the breeding birds under test. There is, however, disagreement as to what constitutes a representative sample. One point of view is that all of the daughters of a given mating hatched during the breeding season should be kept without culling for the whole of the first laying year. A modification of this view, such as practiced under the National Poultry Improvement Plan until the 1939 revision, permits preselection of daughters during the first 60 days of production. A second point of view, brought into practice by many breeders, involves no restrictions with regard to culling. Elimination of unsatisfactory producers under this system may be carried on throughout the year. As a consequence, evaluation of the breeding worth of matings on the basis of only the superior daughters usually results. On the other hand, the advantages of this plan rest in the conduct of breeding work at a reduced cost, due to the saving effected in not having to maintain unprofitable layers and to the possibility of disposal of culls, which would die previous to the end of the year, before their value as market poultry is entirely destroyed.

Recently, Bird and Sinclair[3] examined the results from progeny testing with controlled culling, i. e., culling at a constant level for all matings on the basis of egg production records. They suggested that paper culling—disregarding the birds with the lowest annual records in evaluating the results of a mating—may lead to greater accuracy than if the entire flock is used. Furthermore, actual controlled culling throughout the year was suggested as an economy measure.

Bird and Sinclair compared the production means of survivors of three populations described as A, B, and C which were paper-culled at the end of the year. They present differences between B and C at three levels of culling and between populations A and B at two levels. No data are given on results of controlled culling throughout the year. Since only survivors were considered, their data do not indicate how many of the birds which would have been eliminated at any of the periodic cullings under this plan died before the end of the year.

Since the questions raised by Bird and Sinclair are of great importance, it was considered desirable to examine the possibilities of culling practice with respect to progeny testing. Some caution must be exercised in interpreting results such as these, which are based on

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[2] The assistance of Burt Wetzel in the preparation of some of the tabular material is acknowledged.
[3] BIRD, S., and SINCLAIR, J. W. ON THE VALIDITY OF PROGENY TESTS OF SIRES OBTAINED ON CULLED POPULATIONS OF DAUGHTERS. Sci. Agr. 19: 1–6, illus. 1938.

production records. The number of eggs laid by a hen is the phenotypic expression of the action and interaction of many independently transmitted genes, and an analysis of breeding results on the basis of egg production alone leads to a confounding of these component factors. Hence any results obtained by the study of egg records alone are bound to be of limited value.

Furthermore, culling large proportions of a flock may eliminate profitable as well as unprofitable producers. However, the production necessary for a bird to be profitable depends on cost of feedstuffs and price of eggs as well as on the number of eggs laid. No fixed culling percentage can be expected to retain only the profitable birds under varying flock conditions. In this paper several levels of culling are investigated with respect to their effects on evaluation of the progeny test.

MATERIAL AND METHODS

A population of pullets was selected for this study from the University of California flock of Single-Comb White Leghorns. The only standard of selection used was that at least 50 daughters from each sire were to be available. In this manner 12 sire families were obtained in which the number of daughters varied from 52 to 99. The pullets were hatched in 5 weekly hatches in March and April, all pullets alive at 5 months of age being leg-banded and included in this analysis. Differences between the dams mated to the different sires were not considered. This, of course, means that the progeny test as used here applies not to the evaluation of the breeding worth of the 12 sires used, but to that of the mating as a whole, including both the sire and the respective dams mated to each.

Table 1 presents the production and mortality characteristics of the progeny of the 12 sires studied. It may be seen that the 846 pullets involved averaged 151.7 eggs. If the 36.4 percent of the pullets which died are not considered in this computation the average production rises to 199.2 eggs.

TABLE 1.—*Production and mortality of the progeny of the different sires studied*

Sire	Daughters	Mortality of daughters	Production index (eggs)	Production of survivors (eggs)
	Number	*Percent*	*Number*	*Number*
G14	87	25.3	178.2	213.8
G36	79	55.7	93.1	152.3
G52	62	27.4	190.3	225.4
H8	61	36.1	158.0	203.1
H33	52	21.2	162.8	191.2
H42	56	60.7	114.0	193.2
H43	60	38.3	135.4	179.8
H46	68	60.3	111.4	189.5
H62	66	30.3	177.7	226.9
H79	99	24.2	168.7	192.9
H90	95	26.3	165.6	196.8
H91	61	41.0	153.6	203.7
Total	846	36.4	151 7	199.2

The flock was kept intact for the whole year of laying. No culling was actually done, and all of the analyses were carried out after the completion of the year. In this manner it was possible to apply different systems of culling to one and the same population.

The different systems of culling investigated are listed in table 2. System A presupposes no culling whatsoever and the use of the production index (Taylor and Lerner)[4] obtained by dividing the total number of eggs produced from the first egg to September 30 of the year following the year of hatch by the original number of pullets. Instead of this index the other systems take into consideration only the production of birds which survived to October 1 of their second year of life. System B again involves no culling, while systems C, D, E, F, and G designate respectively the culling of the 12, 18, 24, 30, and 36 percent of each sire family which are lowest in egg production The subscripts indicate the frequency of culling during the year. Thus the subscript 1 denotes paper culling on October 1, at the end of the laying year under consideration. Subscripts 3, 4, and 6 indicate the given number of cullings throughout the year. As shown in table 2, on the first of each of the particular months designated, the lowest 2, 3, 4, 4.5, 6, and 8 percent (depending on the total level of culling in each system) of the original number of pullets in each sire family were considered as having been culled. Only birds alive on each particular date were thus treated, the culling being in addition to mortality. All of the culling was based on the production up to the particular date on which the culling was presumed to have taken place.

TABLE 2.—*Description of culling systems*

System	Proportion culled	Culling procedure	Birds remaining after final culling
	Percent		*Number*
A	0	All birds banded considered	846
B	0	Only survivors considered	538
C_1	12	Paper-culled in October	439
D_1	18	do	386
E_1	24	do	335
F_1	30	do	283
G_1	36	do	234
C_3	12	3 cullings of 4 percent each (February, June, October)	475
D_3	18	3 cullings of 6 percent each (February, June, October)	438
E_3	24	3 cullings of 8 percent each (February, June, October)	402
C_4	12	4 cullings of 3 percent each (January, April, July, October)	482
D_4	18	4 cullings of 4.5 percent each (January, April, July, October)	451
E_4	24	4 cullings of 6 percent each (January, April, July, October)	412
C_6	12	6 cullings of 2 percent each (December, February, April, June, August, October).	486
D_6	18	6 cullings of 3 percent each (December, February, April, June, August, October).	461
E_6	24	6 cullings of 4 percent each (December, February, April, June, August, October).	432

It should be clearly understood that each sire family was culled independently of any other family, so that the level of culling in a given system was the same for each sire family. Systems F_1 and G_1 were not complemented with systems of continual culling at the same total level, because, in the case of some families at least, such heavy culling plus the mortality that occurred would have reduced the families to the vanishing point. The total number of daughters involved under each system is also given in table 2. It may be noticed that some discrepancies in number of birds appear between systems in which the total amount of culling was the same. The reason for this, of course, lies in the fact that some of the birds culled on the

[4] TAYLOR, LEWIS W., and LERNER, I. MICHAEL. BREEDING FOR EGG PRODUCTION. Calif. Agr. Expt. Sta. Bul. 626, 48 pp., illus. 1938.

basis of their low production early in the year died before they were eligible for culling under another system.

The analyses of the above systems were designed to test whether culling fulfills either of its two possible purposes in breeding flocks: (1) To increase the precision of progeny testing by increasing the significance of differences between means of sire families while preserving the relative order of ranking, and (2) to effect managemental economies.

The fulfillment of the first of these purposes may be examined by comparing the variability between and within sire families under the different culling systems. The ratio of the variance between to that within sire families provides a convenient criterion. It is, however, necessary to supplement this measure with a check on the rank order of these sire families. The fulfillment of the second purpose may be examined by the actual comparison of hen-months involved under each system, together with the consideration of the salvage value of the culls, which would have died before the expiration of the year.

The analyses made follow this outline. The only difficulty in interpreting the results lies in the necessity of assuming a normal distribution in each of the sire families, without which a precise study of the variance is not possible. While normal distributions in general are not the rule, this assumption may be justified to a certain extent by the following considerations. Whether or not culling is practiced, the determination of the superiority or inferiority of any given mating has to be made by a comparison of the mean production of this mating with other matings. Truncation (elimination of a part of the curve) and reconstruction of a normal curve from the truncated distribution will obviously yield a value different from the observed mean production. Assumption of normality introduces a decided error in variance analysis but does not lend greater bias to any mating over any other than does the truncation. It is, perhaps, possible to normalize the frequency curves by the choice of suitable coordinates, but in any case these coordinates may be variable from mating to mating. Furthermore, the labor involved and the statistical training necessary for this would preclude any possibility of using such methods in practical breeding work.

RESULTS

PRECISION OF PROGENY TESTS

The mean production of the flock, the variances between and within sire families, and the ratio of these variances for each of the culling systems investigated are shown in table 3. It may be seen that system A exhibits the largest variance both between and within sire families, the ratio of the mean squares also being the largest. Under this system, it will be recalled, mortality and production are evaluated together, so that the net efficiency of the different matings is compared. When production of survivors only is considered, as in all of the other systems, both the variance between and the variance within sire families show a precipitous drop. However, when paper culling only is considered (systems with subscripts of 1), the former drops to a greater extent than the latter. This is shown by the reduction in the ratio of mean squares as compared to the ratio observed under system A. So far as comparison with system B (no culling) is concerned, there is little difference in the ratio of mean squares in the

single culling systems, except for F_1 and G_1 which show a considerably higher variance ratio. They are still greatly below the ratio obtained with system A. More frequent culling increases this ratio over that obtained with system B, but in no case is it as high as the ratio of 11.56 of system A.

TABLE 3.—*Statistical constants of different culling systems*

(1) System	(2) Mean production	(3) Mean square between sires	(4) Mean square within sires	(5) Ratio of mean squares (column 3: column 4)	(6) Number of significant differences between sires
A	151.7	79,571	6,883	11.56	32
B	199.2	16,506	3,286	5.02	24
C_1	221.2	8,668	1,579	5.49	32
D_1	228.8	8,829	1,624	5.44	27
E_1	235.8	7,311	1,810	4.04	20
F_1	239.5	5,689	647	8.79	28
G_1	245.0	4,695	591	7.94	26
C_3	212.9	13,442	1,743	7.71	34
D_3	218.7	12,169	1,392	8.74	34
E_3	224.2	9,667	1,131	8.55	33
C_4	210.5	16,388	1,979	8.28	32
D_4	215.5	15,575	1,658	9.39	35
E_4	222.1	11,144	1,254	8.89	35
C_6	206.6	17,778	2,502	7.11	28
D_6	211.0	20,131	2,072	9.72	36
E_6	215.9	18,322	1,737	10.55	37

Perhaps a more pertinent manner of comparison is to calculate the number of significant differences between the various sire families in each system, the determination of such significance to be made on the basis of the standard deviations and number of degrees of freedom available from each mating. With 12 sire families, if every family is significantly different from every other, 66 significant dfferences would be present. The last column of table 3 shows the number of such dfferences found, based on the actual degrees of freedom available in each mating under each system. They range from 20 for system E_1 to 37 for E_6. It is apparent that single culling on the whole shows the lowest number of significant differences, while more frequent cullings, on the other hand, tend to show a greater number of such differences.

TABLE 4.—*Comparative amounts of useful differentiation available in each culling system*

System	Significant differences as percent of total possible differences	Differentiation when quartiles are based on all birds			Differentiation when quartiles are based on survivors		
		High quartile	Low quartile	High +low	High quartile	Low quartile	High +low
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
A	48.5	42.4	75.8	59.1	42.4	69.7	56.1
B	36.4	45.5	54.5	50.0	45.5	57.6	51.6
C_1	48.5	54.5	42.4	48.5	54.5	54.5	54.5
D_1	40.9	45.5	27.3	36.4	45.5	45.5	45.5
E_1	30.3	42.4	12.1	27.3	42.4	36.4	39.4
F_1	42.4	60.6	15.2	37.9	60.6	33.3	47.0
G_1	39.4	57.6	12.1	34.9	57.6	30.3	44.0
C_3	51.5	57.6	51.5	54.6	57.6	57.6	57.6
D_3	51.5	63.6	51.5	57.6	63.6	57.6	60.6
E_3	50.0	60.6	42.4	51.5	60.6	48.5	54.6
C_4	48.5	57.6	48.5	53.1	57.6	60.6	59.1
D_4	53.0	60.6	54.5	57.6	60.6	66.7	63.7
E_4	53.0	63.6	51.5	57.6	63.6	60.6	62.1
C_6	42.4	54.5	45.5	50.0	54.5	57.6	56.1
D_6	54.5	72.7	57.6	65.2	72.7	63.6	68.2
E_6	56.1	63.6	57.6	60.6	63.6	69.7	66.7

The nature of the differences observed is analyzed in table 4. The second column shows the numbers listed in the last column of table 3 as percent of the total possible differences (66). These percentages can be broken down by consideration of the section of the distribution of the means of sire families in which the significant differences occur. The particular utility of differentiation between sire families lies in the upper limits of the distribution (selection of superior matings) and in the lower limits (elimination of inferior matings), rather than in the midportion of the distribution. Hence, the differentiation of the upper and lower quartiles from the other families may be designated as useful differentiation.

While the different systems may be compared among themselves, it is desirable to designate the quartile order of sire families in one system as a standard in order to be able to pass judgment with regard to such differentiation. System A, based on the production index, measures the actual efficiency of production, taking both egg production and mortality into consideration. Hence it was used as a base. For purposes of distinguishing the factors determining egg production from those affecting mortality, system B, involving only survivors, was chosen as another base.

The percentages of total possible useful differentiation were thus calculated for each of the systems on two bases, that of ranking with regard to the production index (all birds) and to the production of survivors. These percentages are shown in table 4. Once more multiple cullings show an increase in percentage of differentiation over that observed in single cullings. However, the differences between the culled and unculled groups are found to be largely in the differentiation of the upper quartile of sire families. In the lower quartile, system A shows a considerably higher percentage of differentiation. In other words, culling as here investigated makes it easier to select out the better matings, but makes it harder to distinguish the inferior matings.

The next step involves the question of preservation of the rank order of the different sire families in each of the culling systems. As in table 4, systems A and B served as the two bases. Table 5 presents

TABLE 5.—*Coefficients of rank-order correlation between sire families in each system*

System	Correlated with system A		Correlated with system B		System	Correlated with system A		Correlated with system B	
	ρ	ρ^2	ρ	ρ^2		ρ	ρ^2	ρ	ρ^2
B	0.779	0.61	1.000	1.00	E_3	.476	.23	.782	.61
C_1	.538	.29	.860	.74	C_4	.573	.33	.845	.71
D_1	.441	.19	.762	.58	D_4	.552	.30	.831	.69
E_1	.378	.14	.734	.54	E_4	.476	.23	.775	.60
F_1	.291	.08	.650	.42	C_5	.577	.33	.870	.76
G_1	.238	.06	.622	.39	D_5	.650	.42	.851	.72
C_3	.497	.25	.796	.63	E_5	.497	.25	.796	.63
D_3	.448	.20	.747	.56					

coefficients of rank-order correlation of sire families between each of the systems and the two bases, namely, all birds (A) and survivors (B). It will be seen that the correlation coefficients tend to drop with increased culling. Less than 45-percent determination (ρ^2) is available, when comparison of the systems involving culling is made with system A, system G_1 falling as low as 6 percent, while system B shows

over 60-percent determination. The coefficients for the culled groups are considerably higher when comparison with system B is made.

It may thus be seen that a high ratio between mean squares, such as observed in system E_6 (table 3) is not in itself a guarantee of increased precision of differentiation between sires, since in this particular instance the rank order of sire families bears only little relation to that in the unculled population (table 5). Conversely, a low ratio, such as is found in system B may still be associated with a high coefficient of rank-order correlation.

ECONOMIES DUE TO CULLING

The systems that involve culling at the end of the year do not effect any economies. On the other band, systems of culling three, four, and six times a year produce twofold economies: (1) The salvage meat value of the culled birds, which otherwise would have died in the course of the year, and (2) the saving on feeding of the culled birds. Table 6 presents estimates of such economies for this flock. It may

TABLE 6.—*Economy effected under each system of culling*

System	Number of birds	Culls salvaged as proportion of—		Saving on feeding	
		All dead birds of system A	All culls of each system	Hen-months	Percent of all hen-months
		Percent	*Percent*	*Number*	*Percent*
C_3	35	11.36	35.35	360	3.55
D_3	51	16.56	33.55	467	4.60
E_3	67	21.75	33.00	628	6.19
C_4	43	13.96	43.44	407	4.01
D_4	66	21.43	43.42	609	6.00
E_4	76	24.68	37.44	759	7.48
C_6	47	15.26	47.47	695	6.85
D_6	76	24.68	50.00	937	9.23
E_6	97	31.49	47.78	1,118	11.01

be seen that under the 12-percent culling system, 11 to 15 percent of the birds dying annually in the unculled flock can be converted into market hens. Under the 18-percent culling system, these figures rise to 16 to 25 percent; and with 24-percent culling, from 21 to 31 percent of the birds which would die before the end of the first year of production can be sold for meat. This represents from 33 to 50 percent of all of the culled birds, according to the amount and frequency of culling.

The saving in feed and other expenses coincident with the keeping of the unprofitable producers for the whole year varies from 3 to 11 percent of the total hen-months, again according to the amount and frequency of culling.

PRECISION OF LIVABILITY TESTS

Table 7 presents the correlation coefficients of mortality rank order under the various culling systems. Again, paper culling at the end of the year does not affect these ranks. It may be seen that the correlations obtained between the nonculled and culled sire families are all above 0.92, when total mortality is considered, and above 0.93 for mortality of neoplastic origin. This suggests that selection against

high mortality can be carried on with practically the same degree of efficiency under the systems of culling investigated here as on a non-culled population.

DISCUSSION AND CONCLUSIONS

The work of Bird and Sinclair [5] seems to be the only investigation to date concerning the effect of culling on the progeny test. As already mentioned, these workers examined the records of survivors of three populations of pullets of unspecified origin. The data presented here differ from their material both in scope and in the methods of analysis. Here, comparisons are made of 12 populations of pullets originating from 12 different sires. Mortality was taken into account both in relation to the precision of the progeny test and in relation to managemental economies. The birds whose records were studied were hatched in a period of 4 weeks and carried through the first laying year in a manner closely approaching that used in practical breeding work. The application of the statistical methods employed has been discussed previously in the presentation of results.

TABLE 7.—*Coefficients of rank-order correlation with respect to mortality under different culling systems*

System	Correlation with actual mortality		System	Correlation with actual mortality	
	Total	Neoplastic		Total	Neoplastic
C_3	0.958	0.965	E_4	0.972	0.965
D_3	.923	.965	C_6	.972	.979
E_3	.923	.965	D_6	.972	.972
C_4	.951	.972	E_6	.965	.937
D_4	.965	.965			

Tables 4 and 5 may be considered to summarize the findings with regard to the comparative efficiency of segregation of superior and inferior sire families under systems with and without culling. It is apparent that the total percentage of useful differentiation based on all birds is not increased in the single culling systems over that of system A. Furthermore, the coefficients of rank-order correlation are of comparatively low magnitude both for single and multiple culling systems. Systems of culling throughout the year yield much higher percentages of useful determination in the upper quartile of the distribution of egg production means, but fall below that of system A in the lower quartile. At best they equal system B. Only systems D_6 and E_6 exceed the total percentage of useful determination obtained under system A. However, even with these it may be seen from table 3 that the ratio of mean squares between to that within families is still the highest under system A. So far as the coefficients of rank-order correlation with respect to mortality are concerned, very high values are obtained in all of the systems considered.

The economies as observed in the flock exceed 10 percent of feeding costs only in one case (that of system E_6), while up to approximately 30 percent of the birds dying annually can be salvaged for market-meat purposes under systems of culling throughout the year.

[5] See footnote 3.

In drawing conclusions from the data here presented, a possible adverse effect of culling practices on the measurement of individual inherited characters, which are components of the annual egg record, has not been considered. Before any degree of culling of breeding flocks may be advocated without qualification, this possibility should be eliminated.

The ultimate answers to the questions raised in this paper may be found only in actual breeding tests of birds selected under the different systems. However, from the data here presented it may be concluded that, when annual egg production and livability are the gross desiderata considered:

(1) Paper culling at the end of the year is of questionable value.

(2) No system of culling investigated in this paper gives greater differentiation between matings than the system used in the calculation of the production index, which involves both production and mortality characters.

(3) Controlled culling throughout the year at levels of 12 to 24 percent enhances the possibilities of selecting the sire families adjudged superior by the production index, at the same time decreasing precision in the elimination of inferior families.

(4) Such systems of culling do not affect to any great extent the accuracy of determination of the rank order of sire families with respect to first-laying-year mortality.

(5) Economies effected by culling in the flock include 3 to 11 percent of the feeding cost and the conversion into market meat of 11 to 31 percent of the birds which would die during the first laying year.

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ACTION OF SOME ORGANIC COMPOUNDS ON YIELD, SPORULATION, AND STARCH FORMATION OF ASPERGILLUS NIGER[1]

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INTRODUCTION

Studies dealing with the action of organic compounds on the metabolism of organisms have assumed considerable importance, and much activity now exists in the fields concerned with vitamins, hormones, therapeutic compounds, insecticides, fungicides, and compounds capable of inducing carcinomas. Relatively few such investigations, however, have concerned themselves with *Aspergillus niger* Van Tiegh., though it is one of the two fungi whose nutrition is best understood.

The compounds selected for study were therefore chosen for different reasons. The effects of the 37 biological stains are of interest because of their use as "vital" stains, their toxicity, and their property of fluorescence for which claims have appeared in the literature (*7*).[2] The 85 phenanthrene derivatives were included for somewhat similar reasons and particularly because of their similarity in chemical structure to morphine and certain animal hormones. Miscellaneous compounds, to the number of 21, included alkaloids, pharmaceutical preparations, cancer-producing derivatives, and a few dyes of complicated structure. The effects of these 143 compounds have been observed on yield, sporulation, formation of starch, and ability to produce mutants. The genetical studies, however, have been reported elsewhere (*10*).

The data throw additional light on the subject of "chemical stimulation" of plants (*9*) and the antagonistic relation claimed to exist between growth and reproduction. Observations dealing with the effects of low sucrose, low nitrogen, or low trace elements on the action of the substances studied should also prove of interest in this connection.

Little is known concerning the conditions requisite for the formation of starch from sugar by fungi. Lappaleinen (*5*) considered starch a normal constituent formed under good nutritional conditions. According to Boas (*1*), however, starch is a pathological product resulting from the destruction of diastase under the influence of high acidity. The presence of ammonium salts was considered to favor the formation of starch because of its physiological acidity. Starch was found by Boas to be formed from sugars, higher alcohols, and organic acids. Schmidt (*6*) found that nitrates inhibit starch formation. In the opinion of Chrzaszcz and Tiukow (*2*), the elaboration of starch by fungi is characteristic of low-acid producers. Forty-five species were studied.

[1] Received for publication April 1, 1940.

[2] Italic numbers in parentheses refer to Literature Cited, p. 773.

EXPERIMENTAL METHODS

Cultures of *Aspergillus niger* (No. 4247W in the collection of Dr. Charles Thom, of the Bureau of Plant Industry) were grown for 4 days at 35° C. in 200-cc. pyrex Erlenmeyer flasks containing 50 cc. of nutrient solution. The composition per liter of the basic nutrient solution was as follows: Sucrose, 50 gm.; ammonium nitrate, 2.00 gm.; dipotassium phosphate, 0.35 gm.; and magnesium sulfate ($7H_2O$), 0.25 gm.; iron, zinc, copper, manganese, and molybdenum, 0.30, 0.20, 0.05, 0.03, and 0.02 mg. per liter, respectively. Each constituent in this solution was adjusted for maximum yield. Reagent chemicals were used in preparing the solution. The water was redistilled in a pyrex glass still. The sucrose had an ash content of about 0.002 percent. Flasks were sterilized in the steamer for 20 minutes, and inoculation was effected with a spore suspension.

The cultures were filtered, when harvested, with fritted glass crucibles of No. 3 porosity. To indicate the presence of starch, a drop of iodine (N/20) was placed on the reverse of the mycelial felts before they were washed on the filter. It was necessary to wait an hour or more before deciding on the presence or absence of starch. Examination at a 10× magnification was usually advisable. The crucibles containing the mycelial felts were then placed in a current of warm air (45° C.) for 3 to 4 hours, and finally in the oven at 103° overnight. Filtrates from the mycelial felts were also tested for the presence of starch. The quantities of iodine necessary per culture varied considerably under different conditions. No record was made of these variations despite the possibility that ascorbic acid might be concerned therein.

The organic compounds [3] employed in these experiments were each examined under a 5-ampere, black-bulb, mercury-vapor lamp to determine whether they exhibited fluorescence.

EXPERIMENTAL RESULTS

EFFECTS OF BIOLOGICAL STAINS

In table 1 are summarized the results obtained with a number of biological stains when present in the cultures in a concentration of 10 parts per million. The first series was with low sucrose. In no case did addition lead to an increase in growth above that of the control greater than 4.6 percent. The minimum significant variation is considered to be 5 percent. Sporulation generally paralleled growth, but not invariably. Alizarine red S gave a yield of 71.4 percent of maximum, whereas sporulation was depressed to about 12 percent of maximum. Rhodamine B gave identical results on yield and sporulation. The difference in fluorescence between these compounds appeared to be without effect. No general relation, moreover, appears to exist between toxicity, fluorescence, sporulation, or starch formation.

A 50-percent decrease in ammonium nitrate was accompanied in many instances by a diminution in toxicity of these dyes, both with respect to growth and reproduction. The maximum increase in

[3] The phenanthrene derivatives were obtained through the courtesy of Dr. Erich Mosettig, of the U. S. Public Health Service; Federal Security Agency; and the acridine, indanthrone, and indigo derivatives (see table 2), from Dr. W. H. Tisdale, of the Pest Control Research Section, E. I. du Pont de Nemours & Co., Inc.

yield above that of the control was 4 percent. Sporulation usually diminished with decrease in growth, as did also starch. Eosin, erythrosin, and Martius yellow gave cultures with enhanced starch content. Partial nitrogen deficiency in this series had an adverse effect on starch formation.

TABLE 1.—*Effect of some biological stains on growth, sporulation, and starch formation by Aspergillus niger at 35° C. for 4 days in a dibasic optimum solution* [1]

Compound [2] (10 mg. per liter)	Low sucrose (80 percent of optimum)				Low nitrogen (50 percent of optimum)				Low trace elements (50 percent of optimum)			
	Yield [3]	Sporulation [4]	Starch in [5]—		Yield [3]	Sporulation [4]	Starch in [5]—		Yield [3]	Sporulation [4]	Starch in [5]—	
			Mycelium	Solution			Mycelium	Solution			Mycelium	Solution
	Percent				*Percent*				*Percent*			
Control	100.0	8, bl	3	0	100.0	10, bl	3	0	100.0	4, bl	*5*	5
*Acridine orange	34.5	0	5	5	47.3	0	0	0	55.3	0	*5*	5
*Acridine red	93.8	3, bl	3	0	87.0	2, bl	2	0	56.8	0	*5*	5
*Acridine yellow	40.6	0	5	5	69.1	4, bl	0	0	67.7	2, bl	*5*	5
*Acridflavine (neutral)	97.0	8, bl	3	0	97.1	10, bl	0	0	98.3	2, bl	*5*	5
Alizarine blue (Ehrlich)	56.7	2, bl	*5*	5	93.1	2, y	2	2	56.5	2, bl	*5*	5
Alizarine red S	71.4	1, bl	2	0	93.5	4, bl	1	0	73.7	0	*5*	5
Aniline Blue	102.2	8, bl	0	0	97.2	10, bl	3	0	111.4	2, bl	*5*	5
Auramine O	100.4	8, bl	1	0	100.5	10, bl	2	0	104.4	4, bl	*5*	5
Brilliant alizarine	103.2	8, bl	0	0	98.7	10, bl	3	0	119.4	4, bl	*5*	5
Brilliant indigo	91.7	4, bl	5	0	91.2	8, bl	2	0	99.6	4, bl	*5*	5
Chrysoidine	99.1	4, bl	3	0	98.0	10, bl	2	0	94.3	2, bl	*5*	5
*Eosin, W gelb	101.1	2, bl	*5*	0	104.0	8, bl	5	0	112.9	0	*5*	[6]
*Erythrosin bluish	104.6	8, bl	*5*	0	94.6	10, bl	5	0	114.2	2, bl	*5*	[6]
Ethyl red	100.5	8, bl	?	0	100.3	10, bl	3	0	120.3	4, bl	*5*	5
*Fluorescein	99.7	8, bl	?	0	98.7	10, bl	2	9	106.6	3, bl	*5*	5
Fuchsine S	98.8	6, bl	5	0	97.9	10, bl	3	0	116.6	3, bl	*5*	5
*Indigo carmine	102.1	6, bl	5	0	93.0	8, bl	1	0	100.0	2, bl	*5*	5
Malachite green (crystalline)	0	0	0	0	0	0	0	0	0	0	0	0
Martius yellow	86.5	2, bl	3	0	64.7	2, bl	5	0	84.8	0	3	[6]
*Meldola blue	67.3	1, bl	*5*	5	82.7	4, bl	2	2	62.0	0	*5*	5
*Methyl violet	0	0	0	0	0	0	0	0	0	0	0	0
Methylene blue	57.1	1, bl	5	5	78.8	1, bl	1	0	70.3	1, bl	*5*	5
Methylene violet	86.4	6, bl	5	0	90.4	8, bl	3	0	83.4	3, bl	*5*	5
Naphthol green	95.2	4, bl	0	0	92.7	8, bl	2	0	121.2	2, bl	*5*	5
Nile blue sulfate	0	0	0	0	0	0	0	0	0	0	0	0
Orange G	99.8	4, bl	?	0	97.8	10, bl	3	0	154.4	2, bl	*5*	5
Phosphine	102.1	4, bl	5	0	103.3	8, bl	2	0	131.0	2, bl	*5*	2
*Proflavine	97.9	8, bl	3	9	100.0	10, bl	2	0	100.4	2, bl	*5*	5
Quinoline yellow	104.5	8, bl	?	0	101.3	10, bl	3	0	134.2	3, bl	*5*	5
*Rhodamine B	71.4	1, bl	*5*	?	85.6	2, bl	1	0	84.0	2, bl	*5*	5
Rhoduline GO	48.1	1, bl	5	?	84.6	6, bl	1	0	49.1	0	*5*	5
Rose Bengal	93.0	4, bl	5	0	92.1	6, bl	3	0	113.5	2, bl	*5*	3
Safranine Ow	85.9	4, bl	5	0	99.3	8, bl	3	0	76.2	3, bl	*5*	5
Tartrazine	102.2	8, bl	0	0	98.6	10, bl	3	0	141.5	2, bl	*5*	5
Thionine	100.8	8, bl	2	0	101.8	10, bl	2	0	148.0	2, bl	3	[6]
Trypan blue	102.1	8, bl	2	0	97.0	10, bl	3	0	104.8	4, bl	*5*	5
Trypan red	95.7	6, bl	3	0	82.4	6, bl	0	0	63.0	3, bl	*5*	5

[1] Nutrient solution: Water, 1,000 cc.; sucrose, 50 gm.; NH_4NO_3, 2.06 gm.; K_2HPO_4, 0.35 gm.; $MgSO_4 \cdot 7H_2O$, 0.25 gm.; and Fe, Zn, Cu, Mn, and Mo, 0.20, 0.20, 0.05, 0.03, and 0.02 mg. per liter, respectively.

[2] An asterisk * before the name of a compound denotes that it was found to fluoresce in ultraviolet light.

[3] Yield of control culture taken as 100 percent.

[4] Sporulation is indicated on a scale of 0 (sterile) to 10 (black with spores), and spore color by the initial letters of the words black, brown, and yellow.

[5] Starch is indicated on a scale of 0 (none) to 5 (abundant). Tests giving an immediate blue color are indicated by italics.

[6] Erythrodextrin indicated by red color.

The results obtained with an insufficient quantity of trace elements for maximum yield gave perhaps the most interesting results. Many instances of yields greater than that of the control were obtained, ranging up to an increase in maximum yield of 54.4 percent with

orange G. Examples are also to be found of the so-called "stimulation" response, namely, decreased sporulation accompanied by increased growth. Starch formation was abundant with trace-element deficiency, and to such an extent that the filtrates of almost every culture gave a very strong test for starch.

Cultures with 10 mg. per liter of malachite green, methyl violet, or Nile blue sulfate did not germinate within the duration of the experiments. Further tests with 1 mg. per liter gave practically maximum yields with the two last-named compounds though sporulation was decreased by half, and Nile blue sulfate appeared to increase starch formation slightly. The cultures with 1 mg. of malachite green per liter again did not germinate. Guilliermond and Gautheret (*4*) found malachite green quite toxic to roots of wheat seedlings.

EFFECTS OF MISCELLANEOUS ORGANIC COMPOUNDS

The effects of miscellaneous organic compounds (table 2) are quite similar on the whole to those obtained with biological stains (table 1). Comparison of the three series will reveal that here also low nitrogen increased reproduction and decreased the formation of starch, whereas low trace elements resulted in diminished sporulation and a marked increase in starch production. Moreover, only with low trace elements did increases in growth above that of the control occur upon addition of organic compounds.

TABLE 2.—*Effect of miscellaneous organic compounds on growth, sporulation, and starch formation by Aspergillus niger at 35° C. for 4 days in a dibasic optimum solution* [1]

Compound (10 mg. per liter)	Low sucrose (80 percent of optimum)				Low nitrogen (50 percent of optimum)				Low trace elements (50 percent of optimum)			
	Yield	Sporulation	Starch in—		Yield	Sporulation	Starch in—		Yield	Sporulation	Starch in—	
			Mycelium	Solution			Mycelium	Solution			Mycelium	Solution
	Percent				*Percent*				*Percent*			
Control	100.0	8, bl	2	0	100.0	10, br	3	0	100.0	4, bl	*5*	5
Acenaphthene	97.9	6, bl	3	0	100.2	10, br	3	0	101.7	4, bl	*5*	5
Acridine	99.6	8, bl	?	0	99.0	10, br	3	0	106.0	4, bl	*5*	5
Barbituric acid	99.6	8, bl	5	0	98.9	10, br	3	0	94.7	4, bl	*5*	5
Benzidine sulfate	97.8	6, bl	?	0	97.6	10, br	3	0	133.6	4, bl	*3*	5
Caffeine	101.1	8, bl	3	0	92.3	6, br	2	0	89.6	4, bl	*5*	5
Colchicine	102.1	8, bl	3	0	95.6	8, br	3	0	92.3	4, bl	*5*	5
*1, 2, 5, 6-Dibenzanthracene	100.2	8, bl	?	0	96.7	8, br	3	0	92.4	4, bl	*3*	5
Dibrom indanthrone	96.3	8, bl	2	0	98.1	10, br	3	0	116.5	4, bl	*5*	0
*Dibrom indigo	95.6	6, bl	2	0	94.2	8, br	3	0	112.9	4, bl	*5*	5
Dichlor indanthrone	98.4	8, bl	2	0	97.4	8, br	3	0	108.6	4, bl	*5*	5
Digitonin	86.8	4, bl	2	0	81.0	4, br	3	0	80.9	4, bl	*5*	5
Flavanthrone	96.6	8, bl	1	0	96.2	8, br	3	0	124.3	4, bl	*5*	5
Indanthrone	97.0	8, bl	2	0	99.4	10, br	3	0	134.5	4, bl	*5*	5
*Methylcholanthrene	98.9	8, bl	2	0	98.2	10, br	3	0	102.7	4, bl	*5*	5
Monochlor indanthrone	97.5	8, bl	2	0	98.0	8, br	3	0	111.7	4, bl	*5*	5
Phenanthrene	98.0	6, bl	2	0	95.5	6, br	3	0	108.0	4, bl	*5*	5
*Quinine hydrochloride	100.8	8, bl	3	0	98.7	10, br	3	0	103.3	3, bl	*5*	5
Saponin	88.3	2, bl	2	0	88.0	2, br	2	2	91.2	4, bl	*5*	5
Sulfanilamide	97.8	8, bl	2	0	99.3	10, br	3	0	78.6	4, bl	*5*	5
Tetrabrom indigo	92.4	8, bl	3	0	90.3	4, br	2	0	101.9	4, bl	*5*	5
Theobromine	97.3	7, bl	3	0	102.0	10, br	3	0	113.0	2, bl	*5*	5

[1] Footnotes 1 to 5 of table 1 apply also to this table.

EFFECTS OF PHENANTHRENE DERIVATIVES

In the experiments of table 3 the cultures were grown on a low-sucrose solution containing 100 p. p. m. of the different phenanthrene derivatives. It will be noted that the property of fluorescence of the various compounds bore no relation to any of the results obtained. The maximum increase in yield over that of the control amounted to 8.9 percent with compound St 53. Compounds St 8, St 11, St 15, and St 25 also gave increases in weight of over 5 percent. Only derivatives St 14 and St 15 gave increased sporulation; the increase, however, was but slight. The most marked of the increases in starch formation was brought about by St 6 and St 44. Greatly increased starch formation also resulted from addition of compounds St 1, St 14, St 21, St 33, St 36, St 39, St 40, St 41, St 43, St 46, St 48, St 53, St 57, St 58, St 61, St 76, and St 77. Many of the compounds causing increased production of starch were carboxylic acids or hydrochloride salts.

TABLE 3.—*Effect of some phenanthrene derivatives (at 100 mg. per liter) on growth, sporulation, and starch formation by Aspergillus niger at 35° C. for 4 days in a dibasic optimum solution* [1]

Compound No.	Phenanthrene derivative (100 mg. per liter, or 0.01 percent)	Yield	Sporulation	Starch in—	
				Mycelium	Solution
		Percent			
	Control	100.0	8, b1	2	0
St 1	1-oxo-1, 2, 3, 4-tetrahydrophenanthrene	69.0	1, b1	5	0
St 2	4-oxo-1, 2, 3, 4-tetrahydrophenanthrene	0	0	0	0
St 3	3-acetoxy-6-acetylphenanthrene	85.6	4, b1	3	0
*St 4	Phenanthrene-3-aldehyde	83.5	2, b1	4	0
St 5	Phenanthrene-9-aldehyde	93.3	6, b1	4	0
St 6	9-hydroxyphenanthrene-10-aldehyde	52.5	1, b1	5	3
St 7	3-methoxy-phenanthrene-9-carboxylic acid	88.5	4, b1	3	0
St 8	2-propionylphenanthrene	107.0	8, b1	2	0
St 9	2-hydroxy-9, 10-dihydrophenanthrene	0	0	0	0
St 10	2-hydroxy-3, 7-dipropionyl-9, 10-dihydrophenanthrene	101.5	8, b1	3	0
St 11	2-hydroxy-3, 7-di-*n*-butyryl-9, 10-dihydrophenanthrene	106.4	8, b1	2	0
St 12	Phenanthrene	97.5	4, b1	2	0
St 13	9, 10-phenanthrenequinone	3.5	2, b1	0	0
St 14	2-(1-hydroxy-*n*-propyl)phenanthrene	96.3	10, b1	5	0
*St 15	1-hydroxy-2-acetylphenanthrene	105.7	10, b1	4	0
*St 16	4-hydroxydiacetylphenanthrene	99.7	2, b1	2	0
*St 17	4-hydroxydipropionylphenanthrene	102.7	4, b1	4	0
St 18	3-(2-isonitroso-1-oxo-propyl)phenanthrene	103.8	4, b1	2	0
St 19	3-(2-amino-1-hydroxy-*n*-propyl)-phenanthrene hydrochloride	0	0	0	0
*St 20	3-(2-piperidino-1-hydroxy-*n*-propyl)phenanthrene hydrochloride	0	0	0	0
St 21	9-(2-amino-1-oxo-propyl)phenanthrene hydrochloride	79.7	2, b1	5	0
St 22	Phenanthrene 2-carboxylic acid methyl ester	101.2	4, b1	4	0
St 23	Phenanthrene 2-carboxylic acid ethyl ester	99.4	4, b1	2	0
St 24	3-phenanthryl-methanol	59.0	4, b1	5	0
St 25	9-phenanthryl-methanol	106.1	8, b1	3	0
St 26	9-ethyl-phenanthrene	101.6	8, b1	3	0
St 27	2-(1-hydroxyethyl) phenanthrene	92.8	8, b1	3	0
St 28	9-(1-hydroxyethyl) phenanthrene	104.1	8, b1	4	0
St 29	Phenanthrene-9-carboxylic acid	84.5	2, b1	3	0
*St 30	3-acetoxy-4, 5-phenanthrylene oxide	94.7	2, b1	3	0
St 31	3-(1-hydroxy-ethyl) phenanthrene	0	0	0	0
St 32	3-[2-(diethylamino)-1-hydroxy-ethyl] phenanthrene	0	0	0	0
St 33	9-[2-(dimethylamino)-1-oxo-ethyl] phenanthrene hydrochloride	26.9	1, b1	5	0
St 34	3-phenanthryl-acetic acid	9.7	0	0	0
St 35	β-(9-phenanthryl) acrylic acid methyl ester	98.8	6, b1	2	0
St 36	β-(9-phenanthryl)-propionic acid	74.3	0	5	0
St 37	β-(9-phenanthryl) propionic acid methyl ester	89.9	2, b1	4	0
St 38	β-(3-phenanthryl) acrylic acid ethyl ester	87.9	4, b1	3	0
St 39	β-(3-phenanthryl)-propionic acid	73.2	1, b1	5	0
St 40	β-(3-phenanthryl) propionic acid methyl ester	95.3	1, b1	5	0
St 41	β-(3-phenanthryl)-propionic acid hydrazide	89.3	1, b1	5	0

[1] Footnote 1 of table 1 applies here except that sucrose was used at 40 gm. per liter; footnotes 2 to 5 of table 1 apply also to this table.

TABLE 3.—*Effect of some phenanthrene derivatives (at 100 mg. per liter) on growth, sporulation, and starch formation by Aspergillus niger at 35° C. for 4 days in a dibasic optimum solution*—Continued

Compound No.	Phenanthrene derivative (100 mg. per liter, or 0.01 percent)	Yield	Sporulation	Starch in—	
				Mycelium	Solution
		Percent			
St 42	β-(2-phenanthryl) acrylic acid	105.0	8, b1	3	0
*St 43	β-(2-phenanthryl)-propionic acid	42.1	0	5	0
St 44	β-(2-phenanthryl)-propionic acid hydrazide	17.4	0	5	1
St 45	Phenanthrene-9-carboxylic acid dimethylamide	77.2	6, b1	4	0
St 46	Phenanthrene-3-carboxylic acid dimethylamide	67.5	2, b1	5	0
St 47	9-aminomethyl-phenanthrene hydrochloride	0	0	0	0
St 48	Phenanthrene-3-carboxylic acid-[β-(diethylamino)-ethyl]-ester	84.5	2, b1	5	0
St 49	Phenanthrene-9-carboxylic acid-[β-(diethylamino)-ethyl]-ester hydrochloride	75.4	1, b1	4	0
St 50	2-ω-bromo-acetyl phenanthrene	103.6	6, b1	2	0
*St 51	3-ω-bromo-acetyl phenanthrene	4.8	1, b1	0	0
*St 52	2-[3-(1,2,3,4-tetrahydroisoquinolino)-1-oxo-propyl] phenanthrene hydrochloride	97.6	8, b1	1	0
*St 53	3-(3-piperidino-1-oxo-propyl) phenanthrene hydrochloride	108.9	8, b1	5	0
*St 54	3-[3-(1,2,3,4-tetrahydroisoquinolino)-1-hydroxy-*n*-propyl] phenanthrene hydrochloride	1.9	0	0	0
St 55	9-acetyl-*s*-octahydrophenanthrene	94.3	2, b1	4	0
St 56	1,2,3,4,5,6,7,8-octahydrophenanthrene-9-carboxylic acid methyl ester	101.0	8, b1	4	0
St 57	*s*-octahydrophenanthrene-9-carboxylic acid	78.2	2, b1	5	0
St 58	3-ethoxy-4-acetylaminophenanthrene	95.3	4, b1	5	0
St 59	9-ethoxy-10-acetylaminophenanthrene	97.1	6, b1	3	0
St 60	2-propionyl-9,10-dihydro-phenanthrene	104.8	7, br	3	0
St 61	9,10-dihydrophenanthrene-2-carboxylic acid	38.6	0	5	0
St 62	2-[2-(diethylamino)-1-acetoxy-*n*-propyl]-9,10-dihydrophenanthrene hydrochloride	0	0	0	0
St 63	2-[2-(dimethylamino)-1-hydroxy-*n*-propyl]-9,10-dihydrophenanthrene hydrochloride	0	0	0	0
St 64	2-[2-(diethylamino)-1-acetoxy-*n*-propyl]-9,10-dihydrophenanthrene hydrochloride	9	0	0	0
*St 65	3-hydroxyphenanthrene-4-aldehyde	90.0	2, b1	4	0
*St 66	γ-(1-naphthyl)-*n*-butyric acid	1.0	0	0	0
St 67	γ-(2-naphthyl)-*n*-butyric acid	9.0	0	0	0
*St 68	4-hydroxyphenanthrene	0	0	0	0
*St 69	1-hydroxyphenanthrene	0	0	0	0
St 70	2-hydroxyphenanthrene (2-phenanthrol)	0	0	0	0
St 71	Oxazole or 3-hydroxy-4-aminophenanthrene	101.9	8, b1	3	0
*St 72	3-hydroxy-6-[2-(diethylamino)-1-hydroxy-ethyl] phenanthrene hydrochloride	0	0	0	0
*St 73	3-hydroxy-6-[2-(diethylamino)-1-acetoxyethyl] phenanthrene hydrochloride	89.3	2, b1	2	0
*St 74	3-acetoxy-6-[2-(diethylamino)-1-acetoxyethyl] phenanthrene hydrochloride	83.3	4, b1	3	0
St 75	3-dimethylamino-4-hydroxy-1,2,3,4-tetrahydrophenanthrene hydrochloride	84.3	2, b0	4	0
St 76	2-diethylamino-1-hydroxy-1,2,3,4-tetrahydrophenanthrene hydrochloride	87.2	4, b1	5	0
St 77	3-dimethylaminomethyl-4-oxo-1,2,3,4-tetrahydrophenanthrene hydrochloride	93.4	2, b1	5	0
St 78	3-(dimethylaminomethyl)-4-acetoxy-1,2,3,4-tetrahydrophenanthrene hydrochloride	98.5	4, b1	4	0
St 79	3-(dimethylaminomethyl)-4-hydroxy-1,2,3,4-tetrahydrophenanthrene hydrochloride	91.3	4, b1	4	0
St 80	3-diethylaminomethyl-4-hydroxy-1,2,3,4-tetrahydrophenanthrene hydrochloride	78.9	4, b1	4	0
St 81	3-[(1,2,3,4-tetrahydroisoquinolino)methyl]-4-hydroxy-1,2,3,4-tetrahydrophenanthrene hydrochloride	102.4	8, b1	3	0
St 82	1-dimethylamino-1,2,3,4-tetrahydrophenanthrene hydrochloride	82.7	4, b1	4	0
*St 83	3-hydroxyphenanthrene	0	0	0	0
*St 84	9-aminophenanthrene	3.2	1, b1	0	0
*St 85	2-aminophenanthrene	0	0	0	0

The effect of position of substituents in the phenanthrene molecule is illustrated in certain cases (*8*). An oxo group was perceptibly more toxic in the 4 position (St 2) than in the 1 position (St 1). Phenanthrene with a ω-bromo-acetyl group in the 3 position (St 51) was more toxic than with the same group in the 2 position (St 50). Toxicity of 9-aminophenanthrene (St 84) was somewhat less than that of 2-amino-

phenanthrene (St 85). Toxicity (table 4) of the hydroxyphenanthrenes (St 68, St 69, St 70, St 83) appeared somewhat greater with phenolic hydroxyl in the 3 position than in the 1, 2, or 4 position.

Experiments with those phenathrene derivatives sufficiently toxic to prevent germination at a concentration of 100 mg. per liter were repeated at a level of 10 mg. per liter. The results are shown in table 4. Compound St 20 was omitted unintentionally. Derivatives St 68, St 69, St 70, and St 83 were still toxic and barely permitted germination to take place. All 4 compounds were mono hydroxyphenanthrenes. Of the 12 other compounds tabulated in table 4 a total of 8 were amino derivatives, and 2 hydroxy. None of these compounds led to an increase in starch production at a concentration of 10 mg. per liter, with the possible exception of derivatives St 31 and St 32.

TABLE 4.—*Effect of the more toxic phenanthrene derivatives at 10 mg. per liter on growth, sporulation, and starch formation by Aspergillus niger at 35° C. for 4 days in a dibasic optimum solution* [1]

Phenanthrene derivative (10 mg. per liter, or 0.001 percent)	Low sucrose (80 percent of optimum)				Phenanthrene derivative (10 mg. per liter, or 0.001 percent)	Low sucrose (80 percent of optimum)			
	Yield	Sporulation	Starch in—			Yield	Sporulation	Starch in—	
			Mycelium	Solution				Mycelium	Solution
	Percent					*Percent*			
Control	100.0	8, b1	3	0	St 63	98.9	8, b1	2	0
St 2	92.6	4, b1	3	0	St 64	92.1	6, b1	3	0
St 9	71.6	2, b1	3	0	*St 68	.1	0	0	0
St 19	99.9	8, b1	3	0	*St 69	.3	0	0	0
*St 20					St 70	.1	0	0	0
St 31	97.0	4, b1	4	0	*St 72	85.5	2, b1	3	0
St 32	89.6	4, b1	4	0	*St 83	0+	0	0	0
St 47	101.8	8, b1	1	0	*St 85	86.1	1, b1	2	0
St 62	64.9	4, b1	1	0					

[1] See table 3, footnote 1; footnotes 2 to 5 of table 1 apply also to this table.

DISCUSSION

Though under conditions of extreme toxicity the yield, sporulation, and starch formation of *Aspergillus niger* were all diminished, this was not the case at intermediate stages. Response within this range appeared to depend upon the constitution of the toxic substance; and yield, sporulation, and starch varied independently of each other. In some instances growth was but slightly, whereas sporulation was greatly, diminished. In other cases growth and reproduction were injured in equal degree. Both types of response might be accompanied by either an increase or a decrease in starch content. However, no instance was found of a decrease in growth without a corresponding decrease in spore formation. Malachite green appeared to be most toxic of the substances tested and prevented germination at 1 p. p. m.

Addition of none of the compounds led to significant increase in yield over that of the control with the exception of 3-(3 piperidino-1-oxo-propyl) phenanthrene hydrochloride (St 53) and 2-propionyl-phenanthrene (St 8), which produced yields of 108.9 percent and 107.0 percent, respectively. These increases also were probably without significance. Definite increases in yield over that of the control

occurred only with solutions deficient in trace elements, and therefore under conditions in which the inorganic impurities present in the organic compounds could function. The increases were well defined under these conditions and satisfied the criteria of "chemical stimulation." It can be said, therefore, that none of the organic compounds were capable of leading to an increase in yield in an optimum solution for growth.

Starch formation was increased through a partial deficiency of trace elements and decreased by partial nitrogen deficiency. An increase in starch may be brought about by an improvement in nutrition (increase in yield) or through toxicity.

Instances of a diminution in growth accompanied by increased starch formation were given by acridine orange, acridine yellow, alizarine blue (Ehrlich), meldola blue, methylene blue, rhodamine B, rhoduline GO, and phenanthrene derivatives St 1, St 6, St 21, St 33, St 36, St 43, St 44, St 46, St 57, and St 61. Increased starch formation was accompanied in each case by a decrease in sporulation as well as yield. Eosin W gelb, erythrosin bluish, fuchsine S, indigo carmine, phosphine, and phenanthrene derivatives St 14, St 40, St 53, and St 58 gave increases in starch formation without an adverse effect on yield. No fixed relation between spore and starch formation occurred when yields remained maximum. On the other hand no decrease in yield occurred, though formation of starch did not take place, through the action of aniline blue, brilliant alizarine, ethyl red, fluorescein, naphthol green, orange G, quinoline yellow, tartrazine, acridine, benzidine sulfate, and 1, 2, 5, 6-dibenzanthracene. Caffeine and theobromine did not increase starch in *Aspergillus*, though they are reported by Ciamician and Ravenna (*3*) to do so in green plants.

Tests with N/20 iodine would indicate the presence of several constituents in the hyphal cell walls. The mycelium may absorb none of the iodine solution; an immediate brilliant blue color may be formed; or a brown spot may be formed that may or may not become blue later. It is assumed on the basis of these tests that the cell walls comprise a colorless ground stroma similar to that seen under starvation conditions, chitin which causes the brown reaction, and starch. Infiltration with fatty substance may also enter as a factor.

SUMMARY

The effects of 37 biological stains, 21 miscellaneous organic compounds, and 85 phenanthrene derivatives on growth, sporulation, and starch formation of *Aspergillus niger* were studied at concentrations of 1, 10, 20, and 100 mg. per liter. An increase in yield above that of the control occurred only in a solution partially deficient in trace elements and was attributed to the presence of inorganic impurities in the organic compounds. Toxicity was greatest with malachite green (1 p. p. m.), though the hydroxyphenanthrenes (10 p. p. m.) were also quite toxic. Responses in yield, sporulation, and starch formation were not uniform, but were related to the chemical constitution of the compound. Starch formation was decreased by low nitrogen and by certain compounds. Low trace elements led to an increase in starch formation, as did also certain relatively toxic compounds.

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HEMICELLULOSES OF ALFALFA HAY [1]

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INTRODUCTION

Although the literature dealing with the chemical composition of forage plants and hays is extensive, little information is available on the hemicelluloses from these plants and plant materials. Furthermore, many of the analytical data on the hemicelluloses recorded in the literature were obtained by methods now known to give erroneous results. Thus many investigators determined the hemicellulose content by subjecting the plant material (which in many cases had not been previously freed of pectin) to hydrolysis with dilute hydrochloric or sulfuric acid, determining the copper reduction value of the hydrolysate, and calculating the result as percentage of dextrose. Others have used the yield of furfural obtained by distilling the plant material with 12-percent hydrochloric acid as a measure of the hemicellulose content. Both these methods give results that are in effect the sum of several factors of unequal magnitude. Thus, on the one hand, the copper-reduction values of the several hexoses, pentoses, *d*-glucuronic acid, and *d*-galacturonic acid vary considerably, and, on the other hand, the yields of furfural afforded by arabinose, xylose, and the hexuronic acids differ greatly. Among the investigators who have used the analytical methods referred to above are Nelson (*15*),[2] Steinmetz (*31*), Leukel (*14*), and Albert (*1*).

A new approach to the study of the hemicelluloses has been made in recent years by a group of English investigators, among whom may be mentioned Schryver and his coworkers (*10*, *30*), O'Dwyer (*21*, *22*, *23*), Norris (*3*, *4*, *20*), Preece (*25*, *26*, *27*), Norman (*17*, *18*, *19*), and Buston (*7*, *8*, *9*). As a result of their work our knowledge of the chemistry of these complex carbohydrates has been considerably increased. New methods of fractionation and separation have been introduced, and in addition it has been demonstrated that the hemicelluloses are not simply hexosans or pentosans or mixtures of these sugar anhydrides, but that they nearly always contain a uronic acid, which may be either *d*-glucuronic acid or *d*-galacturonic acid.

Employing the technique of extraction and fractionation introduced by O'Dwyer (*21*, *22*, *23*), by Norris and coworkers (*3*, *4*, *20*), and by Preece (*25*, *26*, *27*), Burkhart (*6*) isolated two hemicelloulses from alfalfa roots. On hydrolysis both fractions yielded *d*-glucose and *d*-xylose. A uronic acid was also present, but was not definitely identified.

The object of the investigation reported in this paper was to determine the character and composition of the hemicelluloses of alfalfa hay by methods recently developed.

[1] Received for publication November 18, 1939.
[2] Italic numbers in parentheses refer to Literature Cited, p. 779.

METHODS AND RESULTS

The alfalfa (*Medicago sativa*) hay used in this investigation was grown at the United States Department of Agriculture Beltsville Research Center. Analysis [3] showed that it had the following constituents (all results were calculated on material oven-dried at 105° C.): Ash, 8.85 percent; alcohol-benzene extractives, 11.82 percent; furfural, 7.80 percent; uronic acids (as anhydride), 11.66 percent.

The alfalfa was cut into pieces about 1 inch long and extracted for about 30 hours with a 1:2 ethanol-benzene solution. The extracted material was placed in a large evaporating dish, and the residual ethanol-benzene evaporated on the steam bath.

EXTRACTION WITH 0.5-PERCENT AMMONIUM OXALATE SOLUTION

To 600 gm. of ground and ethanol-benzene-extracted alfalfa (563 gm. of moisture-free material), 4,500 cc. of 0.5-percent ammonium oxalate solution was added, and the mixture was digested at 85° C. for 24 hours. From time to time the mixture was stirred manually. The reaction mixture was filtered, and the digestion with ammonium oxalate solution was repeated three times. The combined filtrate from the four extractions was concentrated under reduced pressure to approximately one-fourth of its original volume, and four volumes of 95-percent ethanol was added to it. The precipitate was allowed to settle, was filtered, and washed with ethanol of graded strengths.[4] The yield of moisture-free material amounted to 94.1 gm. (16.7 percent, calculated on the ethanol-benzene-extracted, oven-dried alfalfa). An amorphous grayish product was obtained.

In another experiment 600 gm. of ethanol-benzene-extracted alfalfa was first digested with water at 85° C. for 24 hours and then with 0.5-percent ammonium oxalate solution as described above. The aqueous and the ammonium oxalate extracts were concentrated under reduced pressure, and then each was treated with four volumes of 95-percent ethanol. The crude water-soluble and ammonium oxalate-soluble pectins were filtered off and washed with ethanol of graded strengths. The yields of the water-soluble and the ammonium oxalate-soluble pectins amounted to 23.6 and 72.7 gm., respectively, of moisture- and ash-free material.

To 359 gm. of pectin-free material (equivalent to 340 gm. of moisture-free material) a sufficient quantity of alcoholic sodium hydroxide solution (20 gm. of sodium hydroxide, 400 cc. of water, and sufficient 95-percent ethanol to make 1 liter of solution) was added to cover completely the extracted alfalfa, and the mixture was then digested at room temperature for 24 hours. From time to time the reaction mixture was stirred manually. The plant material was filtered off, and the digestion with alcoholic sodium hydroxide solution was repeated three times. The combined extract was neutralized with hydrochloric acid, and the ethanol was distilled off under reduced pressure. The residual solution was made acid with hydrochloric acid, and the precipitated lignin was filtered off and dried in vacuo at 56° C. over P_2O_5. The yield was 28 gm. The lignin obtained was

[3] Ash was determined by igniting the sample in an electric muffle at 600° C. The ethanol-benzene extractives were determined by extracting the material with a 1:2 ethanol-benzene solution for 30 hours in a Soxhlet extractor. Furfural was determined by the Tollens-Kröber procedure as described by the Association of Official Agricultural Chemists (*5*). The uronic acids were determined according to the procedure recommended by Dickson, Otterson, and Link (*11*), as modified slightly by Phillips, Goss, and Browne (*24*).

[4] The term "graded strengths" as used in this paper means 70-percent, 85-percent, 95-percent, and absolute ethanol.

light brown. When distilled with 12-percent hydrochloric acid it yielded furfural equivalent in weight to 0.5 percent of the weight of the lignin. The filtrate from the lignin precipitate was neutralized with sodium hydroxide solution and evaporated to dryness on the steam bath, and the residual material was dried in the oven at 105° C. When distilled with 12-percent hydrochloric acid, this material gave negligible quantities of furfural, thus indicating that this method of delignification did not bring about any appreciable loss of hemicelluloses.

ISOLATION OF THE HEMICELLULOSES

The material that had been extracted with alcoholic sodium hydroxide solution was placed on the steam bath, and the ethanol was removed by evaporation. It was then mixed with sufficent 5-percent aqueous sodium hydroxide solution to make a thin suspension and was allowed to digest at room temperature for 24 hours. The reaction mixture was stirred from time to time. At the end of the 24-hour period, the mixture was filtered, and the digestion of the residual material with 5-percent aqueous sodium hydroxide solution was repeated three times. When the combined extracts were acidified with acetic acid, only a slight turbidity was produced, thus indicating the absence of any appreciable quantities of hemicellulose fraction A according to the classification and nomenclature of Norris and his coworkers (*3*, *4*, *20*).

To the solution that had been made acid with acetic acid one-half its volume of acetone was added. On standing, a precipitate settled out. This was separated with the aid of the centrifuge. The product was washed with ethanol of graded strengths, and after centrifuging, the wash ethanol was removed by decantation. The product was dried in the desiccator over calcium chloride. The yield of this fraction, which corresponded to the B fraction according to the classification of Norris and his coworkers, amounted to 8.0 percent of the moisture-free and ethanol-benzene-extracted material.

A small portion of the filtrate from the above-described hemicellulose preparation, to which another one-half volume of acetone was added, failed to yield any precipitate, thus indicating the absence of any C fraction. However, when 1 volume of 95-percent ethanol was added, some precipitate settled out. This precipitate was separated with the aid of the centrifuge and washed with ethanol of graded strengths. The yield amounted to 1.9 percent (calculated on the basis of the moisture-free product and the moisture-free and ethanol-benzene-extracted starting material). This product, which may be designated as the D fraction, contained 6.88 percent of uronic acids (as anhydrides) and when distilled with 12-percent hydrochloric acid afforded 20.38 percent of furfural. Owing to lack of sufficient material, this product was not examined further.

PURIFICATION OF HEMICELLULOSE FRACTION B

Fraction B was purified by the method of Salkowski (*28*). The hemicellulose was dissolved in 4-percent aqueous sodium hydroxide solution, the solution was filtered through glass wool, and the filtrate poured into an excess of Fehling's solution. The bulky and gelatinous hemicellulose-copper complex was filtered off, and dissolved in dilute hydrochloric acid solution. An equal volume of acetone was then added. The precipitated hemicellulose was filtered off and washed

with ethanol of graded strengths. It was dried in the Abderhalden drier over P_2O_5 at 56° C. The yield amounted to 4.3 percent of the moisture-free and ethanol-benzene-extracted alfalfa. Nothing definite was isolated from the filtrate of the hemicellulose-copper complex.

An analysis of the purified B hemicellulose fraction gave the following results (calculated on the moisture- and ash-free basis): Nitrogen, 1.03 percent; total furfural (calculated as pentosans), 77.34 percent; uronic acids (as anhydrides), 12.13 percent; methoxyl, 2.27 percent.

HYDROLYSIS OF HEMICELLULOSE FRACTION B

The hemicellulose (14.5 gm.) was treated with 725 cc. of 2.5-percent sulfuric acid, and the mixture was boiled under the reflux condenser for 6 hours. The dark insoluble material was filtered off. When dried at 105° C. it amounted to 0.88 gm. The filtrate was partly neutralized with a solution of barium hydroxide, and the neutralization was completed with barium carbonate. The barium sulfate and the excess of barium carbonate were filtered off and washed with hot water, and the washings were added to the main filtrate. The filtrate was clarified with Norit and with Filter Cel and concentrated under reduced pressure to a volume of approximately 100 cc. at a temperature not exceeding 50°. To the concentrated solution was added five times its volume of 95-percent ethanol, and the precipitated barium salt was filtered off. When dried in the Abderhalden drier over P_2O_5 at 56° C., this salt weighed 1.26 gm. The filtrate from the barium salt was concentrated under reduced pressure to a thin sirup, and 5 volumes of alcohol was added to it. The solution, which became only slightly turbid, was filtered and concentrated in vacuo to a thin sirup. On standing, crystals separated out and were filtered off and recrystallized from 70 to 75-percent ethanol. The crystals were identified as *d*-xylose by specific rotation, by Bertrand's (*13, p. 58*) method, and by their optical properties.[5] The yield amounted to 10 gm.

A benzylphenylhydrazone and diphenylhydrazone were prepared from the sirup from which xylose had separated. They were identified by their melting points as the hydrazones of *l*-arabinose. The optical properties of the diphenylhydrazone agreed exactly with those of a known specimen of *l*-arabinose diphenylhydrazone.

By means of the Wise and Peterson (*33*) modification of the Neuberg and Wohlgemuth (*16*) method for the quantitative estimation of arabinose, it was found that the B hemicellulose fraction contained 0.8 percent of arabinose.

The sirup was also tested for glucose, fructose, mannose, and galactose, with negative results.

The barium salt (1.26 gm.) that had been precipitated from the sugar solution with 95-percent ethanol gave Tollens' (*32*) naphthoresorcinol test for uronic acid. It gave no music acid on oxidation with nitric acid, and also with bromine water, thus indicating that it was not galacturonic acid. The *p*-bromphenylosazone was prepared according to the procedure of Goldschmiedt and Zerner (*12*). The osazone melted with decomposition, but the decomposition point (184°) was considerably lower than that recorded by Goldschmiedt and Zerner. Although it seems probable that the uronic acid ob-

[5] All identifications by optical methods were made by G. L. Keenan of the Microanalytical Division of the Food and Drug Administration, U. S. Department of Agriculture.

tained was *d*-glucuronic acid, nevertheless, in view of the lower decomposition point of the isolated *p*-bromphenylosazone of the barium salt, this cannot be stated with certainty. The possibility that it may have been one of the methyl ethers of *d*-glucuronic acid is not excluded, especially since the barium salt contained 1.4 percent of methoxyl. Sands and Gary (*29*), as well as Anderson, Russell, and Seigle (*2*), found it difficult to identify the uronic acid in the presence of the methoxyl group.

SUMMARY

The hemicelluloses of alfalfa hay were isolated. When fractionated according to the method of Norris and his coworkers, they were found to consist almost entirely of the B fraction. On hydrolysis this product yielded *d*-xylose, together with a small amount (0.8 percent) of *l*-arabinose. This fraction contained also a uronic acid that was probably glucuronic acid or one of its methyl derivatives.

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HEMICELLULOSES OF WHEAT STRAW [1]

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INTRODUCTION

In 1890, 2 years before the term "hemicellulose" was introduced into chemical literature by Schulze (*12*),[2] Allen and Tollens (*1*) extracted wheat straw with alkali and obtained a product that on hydrolysis afforded *d*-xylose. In 1901 Salkowski (*11*) extracted wheat straw with boiling 6-percent sodium hydroxide solution and showed that the xylan could be precipitated from the alkaline extract by means of Fehling's solution and thus freed of certain impurities. Heuser, Braden, and Kürschner (*6*) improved Salkowski's method, and in order not to contaminate their product with lignin, they used bleached straw pulp (method of delignification not given) as their starting material. They obtained a product that was 96 percent xylan.

The investigation reported in this paper was undertaken for the purpose of determining the character and composition of the hemicelluloses of wheat straw by methods recently developed.

METHODS AND RESULTS

Analysis showed that the wheat (*Triticum aestivum*) straw used in this investigation had the following constituents: [3] Ash, 4.37 percent; ethanol-benzene extractives, 6 percent; pentosans,[4] 29.4 percent; uronic acids (as anhydride), 4.5 percent; pectin (as calcium pectate), 1 percent.

The straw was cut into small pieces and extracted for about 30 hours with a 1:2 ethanol-benzene solution in a large Soxhlet-type extractor. The extracted material was heated on the steam bath until free of solvent and then ground in a Wiley mill until it was fine enough to pass a 60-mesh sieve. The extracted straw was treated with hot (85° C.) water in the proportion of 1 liter of water to 100 gm. of straw, and the mixture was digested at 85° for 4 hours. The straw was filtered off, and the extraction with hot water was repeated twice. The combined aqueous extract was concentrated under reduced pressure and treated with four volumes of 95-percent ethanol. The precipitate was then filtered off. After drying it amounted to 2.1 percent of the weight of the dry ethanol-benzene-extracted straw.

The straw that had been extracted with hot water was then extracted with 0.5-percent aqueous ammonium oxalate solution at 85° C., and the procedure described above for the hot-water extraction was followed, except that 95-percent ethanol that had been acidified with hydrochloric acid was used for the precipitation. The precipitate

[1] Received for publication November 18, 1939.

[2] Italic numbers in parentheses refer to Literature Cited, p. 785.

[3] The analytical methods used were those described by Phillips and Davis (*10*).

[4] Unless indicated otherwise the percentages of pentosans recorded in this paper have not been corrected for the furfural afforded by the uronic acid.

was filtered off and dried in vacuo over sulfuric acid. The yield amounted to 3.1 percent of the weight of the dry ethanol-benzene-extracted straw.

ISOLATION OF THE HEMICELLULOSES

To 500 gm. (moisture-free) of the straw that had been successively extracted with ethanol-benzene solution, hot water, and ammonium oxalate solution, 4.5 liters of 5-percent aqueous sodium hydroxide solution was added. The mixture was digested at room temperature for 24 hours, being stirred by hand from time to time. It was then filtered, and the digestion with 5-percent aqueous sodium hydroxide solution was repeated four times. The cellulose residue from the last extraction operation was washed with water and dried at 105° C. It weighed 270.7 gm.

The combined alkaline extract was treated with an equal volume of 95-percent ethanol, and after being thoroughly mixed was allowed to stand at room temperature for 24 hours. The supernatant liquid was then drawn off, and the hemicelluloses were freed of sodium hydroxide by repeated washing with 95-percent ethanol. The product was suspended in 70-percent ethanol, and concentrated hydrochloric acid was added to the mixture until it was distinctly acid. After the product had stood overnight, the supernatant liquid was drawn off, and the hemicelluloses were repeatedly washed with 70-percent ethanol until free of sodium chloride. It was then washed with ethanol of graded strengths [5] and finally with anhydrous ether. All the washings were done by thoroughly mixing the hemicelluloses with the solvent in centrifuge bottles and then separating the supernatant liquid with the aid of the centrifuge. The yield amounted to 140.0 gm. The analytical data on this product are as follows: Ash, 5.59 percent; lignin, 3.27 percent; uronic acid (as anhydride), 5.07 percent; total furfural (calculated as pentosans), 80.51 percent.

DELIGNIFICATION OF HEMICELLULOSES

The delignification was accomplished by a modification of the procedure used by Van Beckum and Ritter (*14*) for the preparation of holocellulose. Two 56-gm. portions of the crude hemicelluloses were placed in two centrifuge bottles (400-cc. capacity) each containing 56 cc. of water. Each centrifuge bottle was provided with a stopper through which passed an inlet tube extending nearly to the surface of the hemicellulose preparation, and an outlet tube. The hemicelluloses were thoroughly mixed with the water, and the centrifuge bottles were placed in a bath of ice water. A slow stream of chlorine gas was passed through each bottle for 1 hour. Every 6 to 8 minutes the stoppers in the bottles were removed and the contents stirred. At the end of the chlorination period, 300 cc. of 95-percent ethanol was added to each bottle. The contents were thoroughly mixed and then centrifuged. The supernatant ethanol solution was drawn off, and the chlorinated material in each centrifuge bottle was treated with 300 cc. of a 3-percent ethanolamine solution in 95-percent ethanol, and after being mixed was heated in a water bath at 80° C. for 10 minutes. The reaction mixture was allowed to cool to 50° and then was centrifuged. The supernatant liquid was drawn off,

[5] The term "graded strengths" as used in this paper means 70-percent, 85-percent, 95-percent, and absolute ethanol.

and the extraction with ethanolamine was repeated. The extracted hemicelluloses were then washed with 95-percent ethanol and finally with absolute ethanol and with ether. The material was dried in the Vacuum desiccator over concentrated sulfuric acid. The product was colorless and weighed 115.6 gm. The analytical data on the material are: Ash, 1.78 percent; lignin, trace; uronic acid (as anhydride), 5.73 percent; furfural (calculated as pentosans), 83.21 percent.

FRACTIONATION OF THE HEMICELLULOSES

One hundred grams of the hemicelluloses, delignified as described above, was dissolved in a solution containing 60 gm. of sodium hydroxide in 2,250 cc. of water. The alkaline solution was filtered through glass wool, and the filtrate was made slightly acid with acetic acid. Since a preliminary experiment had indicated that fraction A (classification and nomenclature of Norris and Preece *(2, 3, 9)* was present in only small amounts, no attempt was made to separate this fraction. Accordingly, one-half its volume of acetone was added to the acidified solution, and the hemicellulose fraction B together with the small quantity of fraction A was separated with the aid of the centrifuge. The product was washed with ethanol of graded strengths and finally with anhydrous ether. It was dried in the vacuum desiccator over sulfuric acid. The yield amounted to 79.2 gm.

To the filtrate from the previous hemicellulose fraction 95-percent ethanol was added until no more precipitate settled out. This hemicellulose fraction (fraction C) was removed by centrifuging. It was washed with graded strengths of ethanol and finally with anhydrous ether. It was dried in vacuo over sulfuric acid. The yield was 9.0 gm. The analytical data on the material are: Ash, 3.66 percent; uronic acid (as anhydride), 9.64 percent; pentosans, 70.58 percent; methoxyl, 3.74 percent.

It will be observed that the percentages of uronic acid and methoxyl were considerably higher in this hemicellulose fraction than in the A and B fractions. Because of the limited quantity of material available, this fraction was not hydrolyzed.

HYDROLYSIS OF THE COMBINED A AND B HEMICELLULOSE FRACTIONS

To 35 gm. of the hemicellulose preparation, 750 cc. of 2.5-percent sulfuric acid was added, and the mixture was heated on an electric hot plate under a reflux condenser for 15 hours. The reaction mixture was allowed to cool and then was filtered on a weighed filter paper. After drying at 105° C., the insoluble residue amounted to 0.3426 gm. Approximately nine-tenths of the calculated quantity of barium hydroxide solution was added to the filtrate slowly with stirring, while the temperature of the reaction mixture was kept at 40°. An excess of barium carbonate was then added, and the mixture was heated at 70° to 80° for 20 minutes while it was continually stirred. To this mixture 5 gm. of Norit and 3 gm. of Filter Cel were added, and the heating was continued for another 15 minutes. After the mixture had stood overnight, the clear supernatant liquid was drawn off and filtered. The sediment of barium sulfate, barium carbonate, etc., was transferred to centrifuge bottles and centrifuged, and the clear supernatant liquid was drawn off. The residue was

mixed with distilled water and centrifuged, and the supernatant liquid was separated from the sludge. The clear solutions from the two centrifuging operations were combined, filtered, and added to the main filtrate. This solution was concentrated under reduced pressure to a volume of approximately 100 cc. (the temperature of the bath during concentration did not exceed 50°). The concentrated solution was poured into four volume of 95-percent ethanol, and the resulting mixture was centrifuged. The supernatant liquid was drawn off, and the precipitate of the barium salt was washed with absolute ethanol and with anhydrous ether and was finally dried in vacuo over sulfuric acid. The product weighed 0.5 gm.

The supernatant liquid from the barium salt was concentrated under reduced pressure at 50° C., and the thin sirup was again treated with 4 volumes of ethanol. As no more precipitate of barium salt was obtained, the sugar solution was again concentrated under reduced pressure to a thin sirup, and this was allowed to stand in the vacuum desiccator until it started to crystallize. After an appreciable quantity of sugar had crystallized from the solution, the mixture was filtered. The sugar was recrystallized from dilute ethanol, and after it was dried in the vacuum desiccator over sulfuric acid it weighed 15.5 gm. This sugar was identified as *d*-xylose by the specific rotation and melting point and by Bertrand's method (*5*). The optical properties [6] of the double cadmium salt prepared by Bertrand's method were identical with those of the salt prepared by the same method from a known specimen of pure *d*-xylose.

A diphenylhydrazone was prepared from the sirup from which *d*-xylose had separated. It was identified as the diphenylhydrazone of *l*-arabinose by its melting point and by the optical properties of the crystals.

Analysis by the Wise and Peterson (*15*) modification of the method of Neuberg and Wohlgemuth (*8*) showed that the sirup contained 1.27 gm. of *l*-arabinose. The total reducing sugars (calculated as glucose), determined by the Munsen-Walker procedure, amounted to 10.97 gm. The sirup was also tested for glucose, mannose, and galactose, but with negative results.

The barium salt that had been precipitated from the sugar solution with ethanol gave Tollens' (*13*) naphthoresorcinol test for uronic acids. It gave no mucic acid on oxidation with nitric acid or on oxidation with bromine water, thus indicating that it was not galacturonic acid. An attempt was made to prepare the *p*-bromphenylosazone of glucuronic acid according to the procedure of Goldschmiedt and Zerner (*4*), but a brown amorphous product was obtained that did not appear to have the properties of the *p*-bromphenylosazone recorded by these investigators. Because of lack of sufficient material, it was not possible to try other means of identification, and the uronic acid was therefore not definitely identified.

The analytical data on the combined A and B hemicellulose fractions are as follows:[7] Ash, 4.51 percent; nitrogen, 0.06 percent; lignin, none; methoxyl, 1.33 percent; uronic acid (as anhydride), 4.76

[6] All identifications by optical methods were made by G. L. Keenan of the Microanalytical Division of the Food and Drug Administration, U. S. Department of Agriculture.

[7] In calculating the yield of furfural from the percentage of uronic acid anhydride, the assumption was made that the uronic acid was *d*-glucuronic acid. According to Lefèvre and Tollens (*7*), *d*-glucuronic acid affords 19 percent of its weight of furfural. In calculating the percentages of furfural derived from *l*-arabinose and *d*-xylose, consideration was given to the fact that these sugars furnish 75 and 90 percent, respectively, of the theoretical yield of furfural.

percent; total furfural, 55.8 percent; furfural from uronic acid, 0.9 percent; *l*-arabinose, 3.6 percent; furfural from *l*-arabinose, 1.7 percent; furfural from *d*-xylose (by difference), 53.2 percent; *d*-xylose (calculated from furfural), 92.3 percent; molar ratio of uronic acid to *l*-arabinose and *d*-xylose, 1:0.9:23.

Dr. Sterling B. Hendricks of the Bureau of Plant Industry made an X-ray examination of two samples (Nos. 1 and 2) of the hemicelluloses of wheat straw. Sample No. 1 was prepared as described on pages 782 and 783 of this paper. Sample No. 2 was prepared as follows: The residual cellulosic material obtained in the preparation of sample No. 1 was exhaustively extracted with a 10-percent aqueous sodium hydroxide solution at ordinary room temperature. To the alkaline extract an equal volume of 95-percent ethanol was added, and the precipitated hemicelluloses were separated with the aid of the centrifuge. The product was freed of sodium hydroxide by repeated washing with 95-percent ethanol. The hemicellulose precipitate was then suspended in 95-percent ethanol, and concentrated hydrochloric acid was added to the mixture until it was distinctly acid. After the mixture had stood overnight, the supernatant liquid was drawn off, and the hemicelluloses were washed with ethanol of graded strengths and finally with anhydrous ether. The product was dried in the vacuum desiccator over concentrated sulfuric acid. On analysis this product gave the following results: Pentosans, 73.83 percent; uronic acids (as anhydride), 3.75 percent. Hendricks' report was as follows:

> X-ray diffraction photographs were made from two samples of hemicelluloses from wheat straw. Samples for study were kneaded into small pellets which were mounted in the X-ray beam. Both materials gave the usual type of X-ray powder diffraction pattern characteristic of crystalline substances or randomly oriented fibers. However, diffraction patterns of the two samples differed greatly in the positions and intensities of the interference maxima. Fe K radiation was used in the X-ray work.

SUMMARY

The hemicelluloses of wheat straw were isolated, and the product was freed of nitrogen and lignin. When fractionated, the hemicelluloses were found to consist chiefly of the B fraction, together with a small proportion of the C fraction. On hydrolysis the B fraction afforded *d*-xylose, *l*-arabinose, and a uronic acid that was probably glucuronic acid or a methyl derivative of it. The molar ratio of uronic acid to *l*-arabinose and *d*-xylose in this fraction was found to be 1:0.9:23, respectively.

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